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(54) Title: CELL DIVISION AUTOANTIGEN (CDA) POLYPEPTIDES, GENE SEQUENCES AND USES THEREOF

(57) Abstract: The present invention relates to a Cell Division Autoantigen (CDA) and includes polypeptide sequences, gene sequences encoding CDA, vectors containing those sequences, and host cells transformed to contain CDA sequences. The invention also provides antibodies directed against CDA. The invention also relates to the use of CDA as a therapeutic and diagnostic agent for the treatment of cell division associated diseases including cancer. In an aspect of the present invention, there is provided a nucleotide sequence encoding a cell division autoantigen (CDA) said sequence having the nucleotide sequence according to Figure 1 or SEQ ID NO:1 or a fragment, functional equivalent, analogue, mutant or variant thereof. The invention specifically provides nucleotide and amino acid sequences including SEQ ID NO:1 and SEQ ID NO:2 respectively which encode for the CDA molecule. The invention also encompasses antisera and antibodies, including cytotoxic antibodies specific for the CDA polypeptide and/or CDA-derived polypeptides. The invention further encompasses immunogenic, prophylactic or therapeutic compositions, including vaccines, comprising one or more polypeptides. The invention additionally encompassed nucleotide sequences encoding said CDA polypeptide or CDA-derived polypeptides, functional equivalents, analogues, mutants, and variants thereof.



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**CELL DIVISION AUTOANTIGEN (CDA) POLYPEPTIDES, GENE
SEQUENCES AND USES THEREOF**

5 The present invention relates to a Cell Division Autoantigen (CDA) and includes polypeptide sequences, gene sequences encoding CDA, vectors containing those sequences, and host cells transformed to contain CDA sequences. The invention also provides antibodies directed against CDA. The invention also relates to the use of CDA as a therapeutic and diagnostic agent for the treatment of cell division associated diseases including cancer.

10

INTRODUCTION

Cancer is a general term given to a range of diseases, all of which are the result of the uncontrolled division of cells. There are a range of useful chemical entities available to clinicians for the treatment of cancer. Such agents include
15 antimetabolites, DNA binding compounds, alkylating agents and hormones. These substances are usually cytotoxic and therefore also adversely affect normal cells.

An alternative approach to arresting uncontrolled cell division in a mammal is to
20 identify naturally occurring proteins which act specifically to regulate the cell cycle. It is known that the cell cycle is a strictly regulated and complicated chain of events involving many regulatory proteins and pathways. Enormous information exchanges between those proteins and pathways act to maintain a balance between normal and uncontrolled cell growth. Identification of proteins
25 which can act to arrest the growth of S phase cells by preventing the synthesis of the DNA necessary for cell division to occur could lead to use of the protein in cancer therapy. Polypeptides which are part of cell cycle regulatory pathways may be found in the nucleus of rapidly growing cells.

30 A problem faced in finding such specific proteins is that there are many other proteins present in the nucleus of eukaryotic cells, most with no regulatory function.

Accordingly, it is an aspect of the present invention to overcome or at least alleviate some of the problems of the prior art.

SUMMARY OF THE INVENTION

- 5 In an aspect of the present invention, there is provided a nucleotide sequence encoding a cell division autoantigen (CDA) said sequence having the nucleotide sequence according to Figure 1 or SEQ ID NO:1 or a fragment, functional equivalent, analogue, mutant or variant thereof.
- 10 In another aspect of the present invention, there is provided a nucleotide sequence which encodes a CDA polypeptide or CDA-derived polypeptide fragment, functional equivalent, analogue, mutant or variant thereof said polypeptide having a sequence according to Figure 1 or SEQ ID NO:2.
- 15 The cell division autoantigen (CDA) has been found by the applicants to be a nuclear protein that resides primarily in the nucleolus and to a lesser extent in the nucleoplasm of the cell. The function of the protein has been linked to cell division because of its homology to portion of the Leukaemia-associated protein SET which is also involved in cell division control. The CDA has been shown by
- 20 the applicants that it may halt cell growth.

In another aspect of the present invention there is provided an expression vector or cloning vector encoding a cell division auto-antigen (CDA) said vector including the nucleotide sequence according to Figure 1 or SEQ ID NO:1, or a

25 fragment, functional equivalent, analogue, mutant or variant thereof.

The present invention also provides a host cell transformed or transfected with an expression vector or cloning vector including a nucleotide sequence according to Figure 1 or SEQ ID NO:1 or a fragment, functional equivalent,

30 analogue, mutant or variant thereof encoding a CDA polypeptide or CDA-derived polypeptide, functional equivalent, analogue, mutant, or variant thereof.

In another aspect of the present invention, there is provided an amino acid sequence encoding a cell division auto-antigen and having the amino acid

sequence according to Figure 1 or SEQ ID NO:2 or a fragment, functional equivalent, analogue, mutant or variant thereof.

5 In another aspect of the present invention, there is provided an isolated or substantially pure CDA polypeptide encoded by an amino acid sequence or nucleotide sequence according to Figure 1 or SEQ ID NO:2 or a fragment, functional equivalent, analogue, mutant or variant thereof.

10 In another aspect of the present invention there is provided antibodies that specifically bind CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof.

The present invention also includes therapeutic or prophylactic vaccines and pharmaceutical compositions comprising CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof, or antibodies to these polypeptides in a pharmaceutically acceptable carrier. In the vaccines an immunologically effective amount of the CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof may be used.

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In another aspect of the present invention provides antagonists to CDA polypeptides.

25 Antagonists may act to inhibit activity of the CDA polypeptide generally by blocking its action on the cells. The antagonist may also prevent phosphorylation which appears to be important in the activity of the CDA.

30 In yet another aspect of the present invention there is provided a method of controlling cell division, said method including subjecting cells to an effective amount of CDA or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof to control cell division.

In yet another aspect of the present invention, there is provided a method of preventing and treating cancer, said method including providing an effective

amount of CDA or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof to a patient in need, said amount being effective to halt or slow down cell growth.

- 5 In another aspect of the invention there is provided a method of detection of cell cycle activity, said method including measuring the presence of CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof.
- 10 In another aspect of the present invention there is provided a method of increasing cell division, said method including eliminating expression of CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof.
- 15 In summary, the present invention encompasses the CDA polypeptide and CDA-derived polypeptides and methods for making the peptides. The invention also encompasses antisera and antibodies, including cytotoxic antibodies specific for the CDA polypeptide and/or CDA-derived polypeptides. The invention further encompasses immunogenic, prophylactic or therapeutic
- 20 compositions, including vaccines, comprising one or more polypeptides. The invention additionally encompasses nucleotide sequences encoding said CDA polypeptide or CDA-derived polypeptides, functional equivalents, analogues, mutants, and variants thereof.

25 **DESCRIPTION OF FIGURES**

Figure 1 shows the nucleotide sequence and the amino acid sequence of CDA.

- Figure 2a and Figure 2b shows nucleic acid and predicted amino acid sequences of CDA. The predicted amino acids are represented by single letter
- 30 codes with an in-frame stop codon (*). The sequence shows 4 putative nuclear targeting motifs (boxed), two putative cdc2 kinase phosphorylation sites (shaded) with the candidate phosphorylated residue in bold, and polyadenylation signal sequence (bold and italics) located 15 nucleotides

upstream of poly (A) tail. Amino acids in the central and C-terminal tail which are homologous to SET are underlined.

Figure 3 shows immunoblot and immunoreactivity of clone hT4 with the autoimmune serum β -galactosidase fusion protein with estimated molecular weight of 135 kDa (arrow) produced by lambda gt11 clone hT4 (λ gt11 hT4), is immunoreactive with the autoimmune serum, but not with the normal human serum. β -galactosidase protein (120 kDa) produced by wild type lambda gt11 (λ gt 11 WT) is not reactive with the autoimmune serum.

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Figure 4 shows the molecular and structural features of CDA. I, stick diagram showing N-terminal proline rich (Pr) domain (aa 4 - 121), basic domain (aa 180 - 418), C-terminal acidic domain comprising two acidic regions (aa 420-536 and 564-675, respectively), 4 putative nuclear targeting motifs (aa 9-15; 187-203; 404-407 and 411-414) and 2 cdc2 kinase phosphorylation sites (Ser20 and Thr340). II, Kyte-Doolittle hydrophilicity profile showing that CDA1 is generally hydrophilic. III, predicted secondary structure showing regions forming α -helices (Helix), β -pleated sheet (Sheet) and reverse turns (Turns) using the methods of Chou-Fasman (CF) and Robson-Garnier (RG); agreed predictions between these two methods are presented for α -helices (CFRg Trn). β -Pleated sheet (CFRg Sht) and reverse turns (CFRg Trn). IV and V, profiles of acidic (A) and basic (B) amino acid residues of CDA1 and human SET respectively.

15

Figure 5 shows CDA is phosphorylated *in vivo*. Overexpressed myc-tagged full length (A) and N-terminal two thirds (B) of CDA1 are metabolically labelled by ^{35}S -methionine and immunoprecipitated by anti-myc-tag McAb 9E10, and migrate in the SDS-PAGE as apparent molecular weight of 120 kDa (arrow) and 60 kDa (arrow), respectively. They both are labelled by ^{32}P *in vivo* and immunoprecipitate any ^{35}S , nor ^{32}P labelled proteins.

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Figure 6 shows Northern blots illustrating molecular size of CDA mRNA in human testis tissue and HeLa cell lines. RNA blots show a single 2.8 kb mRNA band of CDA in HeLa cells and human testis detected by hybridisation with CDA cDNA probes labelled with ^{32}P -dCTP. Approximately 350ng poly A+ RNA of

Hela cells and human testis were hybridised respectively with probe 1 (PCR fragment from clone hT6 corresponding to nucleotides 1421 - 2280) and probe 2 and 3 (PCR fragments from clone hTsl-9 corresponding respectively to nucleotides 1595 - 2808 and 741 - 1418).

5

Figure 7 shows species distribution of CDA. A: Rabbit antiserum raised against a bacterial fusion protein of GST and CDA1 C-terminus (GST-hT4) is affinity purified by firstly removing anti-GST antibodies followed by a GST-hT4 fusion protein affinity column purification. Whole serum (i) contains both anti-GST (lanes 1) and anti-GST-hT4 fusion protein (lanes 2) antibodies; the flow-through fraction of the first GST-column (ii) with anti-GST antibodies removed retains the anti-GST-hT4 fusion protein antibodies (iii) are reactive with GST-hT4 fusion protein, but not with the purified GST. B: The affinity purified antibodies are reactive with CDA1 of approximately 120 kDa in HeLa and HepG2 cells of human origin, as well as COS cells of monkey. No band around 120 kDa was detected by the antibodies from cell lines of dog (MDCK), mouse (thymus B6TEA and 133.D7), hamster (CHO and BHK) and chicken (SSC). Approximately 30 µg/lane total protein extract was loaded, and the blot was re-blotted with anti-alpha tubuline monoclonal antibody to show equal loading of each lane.

Figure 8A and 8B (1 and 2) shows the effect of various levels of CDA on cell growth. A: Approximately 5,000 cells of the stable cell lines with full length and N-terminus of CDA were mixed with untransfected HeLa cells as supporter or feeder cells and cultured with various concentrations of DOX in the medium. After 2 days hygromycin was added to kill the feeder HeLa cells. The cells were cultured in presence of hygromycin for additional 10 days with the medium changed every 4-5 days. The out-grown cell colonies were fixed in dish and stained with crystal violet before photograph. The DOX concentrations are: 5.00ng/mL (a); 0.50ng/mL (b); 0.25ng/mL (c); 0.13ng/mL (d); 0.06ng/mL (e) and 0.00ng/mL (f). B: Approximately 2×10^5 cells were seeded in each dish (10cm) and cultured in presence of DOX at various concentrations as indicated for 4 days. Cells were labelled with BrdU for 1 hr and stained with anti-BrdU Ab-FITC conjugate and propidium iodide. The dot plot shows the DNA

synthesizing cells content equivalent to S phase cells but are not synthesizing DNA are measured and shown (R4). The viability of the cells determined by Trypan blue exclusion assay is also shown (Viable). The histogram shows the number of cells (counts) against the DNA contents (FL2-A). B1 shows the results for full length CDA and B2 shows results for N-terminus CDA.

Figure 9A and 9B show effect of CDA overexpression on cell growth. A: Equal number of cells (4×10^4) were seeded in 6 well plates and cultured with DOX in the medium overnight. To switch the transgene on, the DOX was removed by replacing the medium and the cells were cultured for 1-5 days. Cells were fixed and stained with 0.1% crystal violet daily. The cell associated dye was then extracted in 10% acetic acid, and the OD (495nm) was measured and plotted against the days. Full length CDA cell line is represented by the faint bar, while the N-terminus cell line by the solid bar. B: Approximately 2×10^5 cells were seed in each 10mm dish, and cultured without DOX to switch on the transgene for 5 days. Cells were labelled with BrdU and harvested daily for FACS scan. The dot plot shows the DNA synthesizing cells incorporating BrdU (FL1 H) and the DNA contents (FL2-A). Percentage of the cells that have DNA content equivalent to S phase cells but are not synthesizing DNA are measured and shown (R4). The viability of the cells determined by Trypan blue exclusion assay is also shown (Viable). The histogram shows the number of cells (counts) against the DNA contents (FL2-A). B1 shows the results for full lengths CDA and B2 shows eresults for N-terminus CDA.

Figure 10 shows the inhibitory effect of CDA on cell growth is disabled by point mutations that abolish the potential cdc2 kinase phosphorylation sites. Approximately 4×10^6 tTa HeLa cells were transient transfected with 15 μ g pTRE construct containing either wild type CDA (Wt) or a double mutant (DM) CDA that has 2 point mutations (S20A and T340A). The transfected cells were cultured for 48 hrs in presence (D+) or absence (D-) of DOX before FACS analysis. The dot plots show the DNA synthesizing cells incorporating BrdU (FL1 H) and the DNA contents (FL2-A). The cells in region 3 (R3) are Synthesizing DNA and have BrdU incorporated. Cells in regions 2, 4 and 5 (R2, R4 and R5) are not synthesizing DNA and have no BrdU incorporated. DNA

contents indicate that the R2 cells have one set of chromosomal DNA and in the stage of G0/G1. R4 cells have double amount of DNA compared to R2, indicating that they are in G2/M phase. R5 cells have DNA content equivalent to S phase cells but not synthesizing DNA, indicating that they are arrested at S phase. The percentage of the cells at each of those status are shown. Note: increased number of R5 cells are seen in Wt D- cells, but not in the mutant transfected cells (DM D-).

Figure 11 shows BrdU incorporation and DNA content in HeLa cells transfected with CDA1-DM (double mutant). Cells (2×10^5 cells per 8 cm dish) of the stable cell line 2C6D3 expressing Myc-tagged CDA1-DM were cultured for 5 days with doxycycline to keep the transgene turned off (Off) or without doxycycline to turn the transgene on (On). On days 4 and 5, cells were analyzed by flow cytometry for DNA content and BrdU incorporation (Figure 11A) and expression level of myc-tagged CDA1-DM by immunoblotting using anti-myc McAb (Figure 11B). Blots were reprobbed with anti- α tubulin McAb to show equal loading of proteins per lane (Figure 11C).

Figure 12 shows interacting proteins of CDA1 in HeLa Cells.

DETAILED DESCRIPTION OF THE INVENTION

In an aspect of the present invention, there is provided a nucleotide sequence encoding a cell division autoantigen (CDA) said sequence having the nucleotide sequence according to Figure 1 or SEQ ID NO:1 or a fragment, functional equivalent, analogue, mutant or variant thereof.

In another aspect of the present invention, there is provided a nucleotide sequence which encodes a CDA polypeptide or CDA-derived polypeptide fragment, functional equivalent, analogue, mutant or variant thereof said polypeptide having a sequence according to Figure 1 or SEQ ID NO:2.

The cell division autoantigen (CDA) has been found by the applicants to be a nuclear protein that resides primarily in the nucleolus and to a lesser extent in the nucleoplasm of the cell. The function of the protein has been linked to cell

division because of its homology to portion of the Leukaemia-associated protein SET which is also involved in cell division control. The CDA has been shown by the applicants that it may halt cell growth.

- 5 The term "functional equivalent, analogue, mutant, or variant thereof" as used herein means a sequence which functions in a similar way but may have deletions, additions or substitutions that do not substantially change the activity or function of the sequence.
- 10 The term "fragments" relates to a portion of an amino acid or nucleic acid sequence that is less than that of full length but is capable of hybridising to a full length nucleotide or amino acid sequence capable of encoding an amino acid sequence of CDA which is a portion of the full length sequence of CDA.
- 15 Throughout this specification the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

Where these terms relate to protein, the changes do not substantially change
20 the activity of the protein. Where it relates to a changed nucleic acid molecule, the change does not result in a change in the reading frame of a protein coding region and preferably encodes a protein having no change, or only a minor reduction in biological function.

- 25 Due to the degeneracy of the genetic code, other nucleic acid sequences which encode the same or functionally equivalent amino acid sequence are included within the scope of the current invention. Such alterations of the nucleotide sequence may include substitutions of different nucleotides resulting in the same or a functionally equivalent gene product. Also included within the scope
30 of this invention are nucleic acid sequences having deletions and/or additions and which result in a functionally equivalent gene product. In addition the gene product may include deletions, additions or substitutions of amino acid residues within the sequence which result in changes that still produce a functionally active product.

The nucleotide sequence of Figure 1 or SEQ ID NO:1 is preferably the full length cDNA sequence of CDA which is approximately 2.8kb long. However, fragments, functional equivalents, analogues, mutants, or variants are also included in the scope of the invention. The full length CDA nucleotide sequence may include a reading frame that encodes 693 amino acids, preferably from nucleotides 204 to 2284 as represented in Figure 1 or SEQ ID NO:1. The mRNA for CDA preferably has an open reading frame of 2079 bp, encoding the predicted polypeptide of 693 amino acids with an apparent molecular weight of 120 kilodaltons and an acidic pI of 4.26.

The CDA nucleotide sequence may include nuclear targeting motifs and/or cdc2 kinase phosphorylation sites as indicated in Figure 2a and 2b. The nuclear targeting motifs aa 9-15 are encoded by nt 153-173; aa 187-203 are encoded by nt 687-737; aa 404-407 are encoded by nt 1338-1349 and aa 411-414 are encoded by nt 1359-1370. The cdc2 kinase phosphorylation sites Ser20 are encoded by nt 186-188 and Thr340 is encoded by nt 1146-.

The present invention also includes fragments of the full length DNA sequence of CDA. The fragments may encode parts of the CDA molecule. The fragments will be derived from the nucleotide sequence of the CDA and will be capable of hybridising to the nucleotide sequence of CDA as represented by Figure 1 or SEQ ID NO:1.

The invention also includes DNA molecules including full length and fragments, functional equivalents, analogues mutants, or variants of the full length that hybridise to the nucleotide sequence of CDA according to Figure 1 or SEQ ID NO:1 or a portion thereof. Hybridisation may be carried out using methods known in the art. For example, hybridisation using the DNA fragments as probes may be carried out in 2xSSC, 1.0% SDS at 50°C and washed using the same conditions.

The full length DNA sequence, functional equivalents, analogues mutants, or variants of the CDA or fragments of the CDA may be synthesised using

methods known in the art or generated via genomic or cDNA libraries once having a source of CDA, DNA or protein. In preparing fragments of the CDA nucleotide sequence, the DNA may be cleaved at various restriction sites using restriction enzymes. Other methods known in the art may be used to generate the fragments.

Preferably the fragments of DNA are overlapping fragments of DNA and are designated as clones hT4 and hT6. These clones were detected and isolated from human testis cDNA lambda gt11 expression libraries.

10

The open reading frame of hT4 and hT6 respectively encoded the C-terminal 142 and 262 amino acids of CDA hT4 starts from the nucleic acid codon for the amino acid 552 (aa552) and hT6 starts from the nucleic acid codon for the amino acid 432 (aa 432) according to Figure 1 or SEQ ID NO:1.

15

The products of the clones were further immunologically reactive with the autoimmune serum from the particular discoid lupus erythematosus (DLE) patients. Figure 3 shows an example that the auto-antibodies react with the β -galactosidase-hT4 fusion protein produced in the recombinant lambda gt11 clone (λ gt11 ht4), but not the β galactosidase itself in the lambda g11 wild type clone (λ gt11 WT), while the normal human serum is not reactive to either of the proteins. The nucleic acid sequences of these clones show that they are overlapping clones with identical sequences at the 3' end both containing a poly (A) tail. The clone hT6 is 360 bp longer than hT4.

25

The DNA of the CDA may be obtained from any biological source including sera, cells or tissue. Preferably the sera contains auto-antibodies to self proteins (autoantigens). The cell lines preferably have increased cell division. The cells may be cancer cells including HeLa cells or Hep G2 or COS cells from the African Green Monkey.

30

In another aspect of the present invention there is provided an expression vector or cloning vector encoding a cell division auto-antigen (CDA) said vector

including the nucleotide sequence according to Figure 1 or SEQ ID NO:1, or a fragment, functional equivalent, analogue, mutant or variant thereof.

5 The DNA containing the full length or fragment, functional equivalent, analogue, mutant or variant thereof of CDA coding sequence may be inserted into an expression vector or cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages
10 such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives. The insertion into a cloning vector can, for example be accomplished by ligating the DNA fragment into the cloning vector or the expression vector which has complimentary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the
15 vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) into the DNA termini; these ligated linkers may comprise specific chemically synthesised oligonucleotides encoding restriction endonuclease restriction sequences. In an alternative method, the cleaved
20 DNA may be modified by homopolymeric tailing.

The nucleotide sequence ligated into the expression vector may be the full length DNA according to the Figure 1 or SEQ ID NO:1, a fragment, functional equivalent, analogue, mutant or variant thereof. The variant may be generated
25 by any of the methods described above and ligated into the vector by methods available to the skilled addressee.

The present invention also provides a host cell transformed or transfected with an expression vector or cloning vector including a nucleotide sequence
30 according to Figure 1 or SEQ ID NO:1 or a fragment, functional equivalent, analogue, mutant or variant thereof encoding a CDA polypeptide or CDA-derived polypeptide, functional equivalent, analogue, mutant, or variant thereof.

Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

- 5 Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors or cloning vectors can be propagated and prepared in quantity.
- 10 Suitable host systems to express the nucleotide sequence may include, but are not limited to, mammalian cell systems infected with virus (e.g. vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g. baculovirus); microorganisms such as yeast vectors, or bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA. Preferably, the bacterium is
- 15 *E.coli*, *B. Subtilis* or *Salmonella*.

In another aspect of the present invention, there is provided an amino acid sequence encoding a cell division auto-antigen and having the amino acid sequence according to Figure 1 or SEQ ID NO:2 or a fragment, functional

20 equivalent, analogue, mutant or variant thereof.

In another aspect of the present invention, there is provided an isolated or substantially pure CDA polypeptide encoded by an amino acid sequence (SEQ ID NO:2) or nucleotide sequence (SEQ ID NO:1) or according to Figure 1 or a

25 fragment, functional equivalent, analogue, mutant or variant thereof.

The polypeptide may comprise 693 amino acids (Figure 1 or SEQ ID NO:2), and has an apparent molecular weight of 120 kDa. Amino acid sequence analysis shows a multiple domain structure consisting of an N-terminal proline rich (Pr) domain, a basic domain homologous to a leukaemia associated

30 protein, and a N-terminal acidic domain (Figure 4). Four putative nuclear localisation signal (NLS) sequences are identified, indicating that CDA1 may reside in the cellular nucleus. Two potential phosphorylation sites by p34^{cdc2} kinase may be present, indicating a role for this protein in cell cycle control.

The protein may be isolated from natural sources or biological sources including sera, cells or tissues or may be recombinantly produced.

- 5 Where the polypeptide is from natural sources, it may be isolated from auto-immune sera containing auto-antibodies to self proteins including self antibodies and self antigens. The autoimmune sera contains autoantigens in free and unbound form, that may bind to the cognate self antigen upon contact. Preferably the sera is obtained from patients having discoid lupus erythematosis (DLE). The polypeptide may also be isolated from cells which divide rapidly
10 such as cancer cells, preferably HeLa cells. Other cell sources include HepG2 cell lines or COS cells derived from the African Green Monkey.

- As used herein the term "isolated and substantially pure" means that the
15 product is significantly free of other biological materials with which it is naturally associated.

- The polypeptide of the present invention may be purified using standard protein or peptide purification techniques including but are not limited to
20 electrophoresis, centrifugation, gel filtration, precipitation, dialysis, chromatography, affinity chromatography, immunoadsorbent affinity chromatography, and gel permeation high performance liquid chromatography, isoelectric focussing, and variations and combinations thereof.

- 25 The protein may be isolated from recombinant sources and may be generated by gene expression systems and host systems as described above.

- Once the polypeptide is expressed standard purification processes may be used to isolate and purify the polypeptide as described above.
30

The present invention further includes CDA molecules with postranslational modifications. Without being limited by theory, studies by the applicants have shown that the antiproliferative activity of CDA may be dependant on its phosphorylation by the kinase p34^{cdc2}. Studies show that point mutations in

CDA to regions containing putative phosphorylation signals lead to the abolition of activity. Therefore a particular embodiment of the present invention includes a CDA molecule which is phosphorylated at one or more amino acid residues. Preferably the phosphorylation is a serine residue or a threonine residue. More preferably the phosphorylation is at Ser20 (aa 20 or S20) or Thr 340 (aa 340 or T340) according to Figure 1 or SEQ ID NO:2.

The phosphorylation status of CDA *in vivo* has been examined by the applicants. Both full length and N-terminus of CDA were expressed in transfected HeLa cells and metabolically labelled with radioactive isotopes ³⁵S and inorganic ³²P followed by immunoprecipitation using anti-myc tag McAb 9E10. Both ³⁵S labelled full length and N-terminus were specifically immunoprecipitated and showed molecular weights of approximately 120kDa and 60kDa, respectively. These are labelled with inorganic ³²P (Figure 5) indicating that CDA is phosphorylated *in vivo*.

The present invention also includes fusion proteins comprising the CDA protein or a fragment thereof and another protein. Preferably the fusion protein is used to immunise rabbits and mice to produce antibodies specific to the CDA. Therefore the other protein may be directed toward improving an immune response to CDA.

Applicants have produced GST fusion proteins with CDA C-terminal fragments using cDNA clones hT4 and hT6. The GST fusion proteins were made using an expression plasmid vector pGEX in *E.coli* and purified on glutathione resin column. The fusion proteins contain GST and CDA (aa 552-693) (GST-hT4) or GST and CDA (aa 432-693) (GST-hT6). The amino acid sequence and residues are according to Figure 1 or SEQ ID NO:2.

In another aspect of the present invention there is provided antibodies that specifically bind CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof.

For the production of such antibodies, isolated or preferably purified preparations of CDA or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof may be used as immunogens. The immunogens may be conjugated to a carrier molecule, preferably a carrier protein. Carrier proteins may be any commonly used in immunology including, but not limited to, bovine serum albumin (BSA), chicken albumin, keyhole limpet hemocyanin (KLH) and the like.

Polyclonal and monoclonal antibodies are included in the scope of the present invention. Immunization regimes for production of both polyclonal and monoclonal antibodies are well known in the art. The immunogen may be injected by any number of routes including subcutaneous, intravenous, intraperitoneal, intradermal, intramuscular, mucosal, or a combination of these. The immunogen may be injected in soluble form, aggregate form, attached to a physical carrier, or mixed with an adjuvant, using methods and materials known in the art. The antibodies may be purified by methods known in the art.

The antibodies of the invention may be used to facilitate isolation and purification of the CDA polypeptide or protein. They may also be used as probes for identifying clones in expression libraries that have inserts encoding CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof. Such antibodies would have use in the purification of CDA molecules during the production of recombinant CDA for example. In a preferred embodiment antibodies are bound to chromatography resins. Passing a protein mixture through the column allows the cognate antigen to adhere. Non-target molecules are flushed through the column to leave purified CDA which is then eluted. In another embodiment antibodies can be used to purify protein by the process of immunoprecipitation. Antibodies can also be used to probe for CDA and closely related proteins in cDNA expressing libraries and cell extracts. The skilled addressee will understand that antibody fragments (such as Fab fragments) will also be useful in the context of the present invention.

The antibodies, particularly those which are cytotoxic, may also be used in passive immunisation to prevent or attenuate rapid cell division, preferably in cancer cells.

- 5 The present invention also includes therapeutic or prophylactic vaccines and pharmaceutical compositions comprising CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof, or antibodies to these polypeptides in a pharmaceutically acceptable carrier. In the vaccines an immunologically effective amount of the
- 10 CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof may be used.

The term "immunologically effective amount" is used herein to mean an amount sufficient to induce an immune response.

15

In yet another aspect of the present invention there is provided a method of controlling cell division, said method including subjecting cells to an effective amount of CDA or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, or variants thereof to reduce cell division.

20

- The cells may be subjected to CDA or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, or variants thereof directly or be genetically manipulated to express CDA or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, or variants thereof in the
- 25 presence of a stimulatory signal which switches CDA production on and off. Alternatively, the cells may be induced to express the *de novo* CDA which has not been introduced by genetic manipulation. Compounds such as doxycyclin may be used to selectively switch CDA expression on and off to over- or under-express the gene. Increased CDA levels may inhibit outgrowth of cell colonies
- 30 and hence inhibit cell growth.

Preferably for its inhibitory function in cell growth, the CDA is encoded by a full length CDA nucleotide sequence or at least, the C-terminus of CDA.

Where CDA is used directly, the protein may be isolated and purified from sources described above or may be recombinantly produced, also as described above.

- 5 The cell division may be controlled by modulating expression and/or activity of CDA or of CDA-derived polypeptides to change the amount of CDA exposed to a cell. By the term "modulating expression and/or activity of CDA" as used herein means modifying or altering the expression and/or activity of CDA compared to unmodified levels. Modulating expression may include increasing
10 or inducing the expression and/or activity or reducing the expression and/or activity.

"Activity" as used herein relates to a function of a CDA in a cell, and includes the ability CDA to bind to chaperone, or upstream or downstream effector
15 molecules thereby activating or repressing upstream or downstream pathways which affect cell division.

Modulation of CDA expression and/or activity in the cell may be achieved using antagonists, inhibitors, mimetics or derivatives of the CDA. The terms
20 "antagonist" or "inhibitor", as used herein, refer to a molecule which, when bound to either CDA, blocks or modulates the activity of CDA. Antagonists and inhibitors may include proteins, nucleic acids, carbohydrates, antibodies or any other molecules including ligands which bind to CDA. Proteins may include enzymes which can break down CDA and therefore affect the level of CDA
25 exposed on the cell. Other modulators of the activity and/or expression of CDA include a range of rationally-designed, synthetic inhibitors.

Modulation of CDA expression and/or activity may be achieved by direct or indirect methods. Modulation of expression and/or activity of CDA may be
30 achieved using direct methods known to those of skill in the art and include, but are not limited to, knockout technology, antisense technology, triple helix technology, targeted mutation, gene therapy, regulation by agents acting on transcription. Indirect methods for modulating expression and/or activity of CDA may include targeting upstream or downstream regulators such as cytokines.

In another aspect of the present invention provides antagonists to CDA polypeptides.

- 5 Antagonists may act to inhibit activity of the CDA polypeptide generally by blocking its action on the cells. The antagonist may also prevent phosphorylation which appears to be important in the activity of the CDA.

10 Antagonists may include antibodies or small peptides directed to the CDA polypeptide or a binding site of the CDA. They may also include mutants or derivatives of CDA which may not act in the manner of CDA but be substantially the same as CDA in physical make-up. Competition experiments may help to identify suitable CDA antagonists. These tests are generally known to the skilled addressee.

15

In yet another aspect of the present invention, there is provided a method of preventing and treating cancer, said method including providing an effective amount of CDA or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, or variants thereof to a patient in need, said amount being
20 effective to halt or slow down cell growth.

Functional studies by the applicants show that increased levels of CDA in a cervical cancer cell line, HeLa, result in cell growth halt. This property of CDA demonstrates its potential to be exploited in cancer control and treatment.
25 Delivery of the CDA to the cancer may be facilitated by introduction of carriers directed to the cancer. This may also be achieved by creating fusion proteins of CDA protein and a cancer specific carrier to direct the CDA to the cancer cell.

In another aspect of the invention there is provided a method of detection of cell
30 cycle activity, said method including measuring the presence of CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof.

CDA is implicated in cell division control. Over expression of the protein may halt cell division. Accordingly, increased cell cycle activity may be measured by an increase in CDA levels. This may be measured directly by measuring the CDA using antibodies, as hereinbefore described, or it may be measured by
5 measuring indirect parameters such as mRNA levels and identifying an increased transcription of CDA protein.

In another aspect there is provided a method of diagnosing cancer, said method including determining a change in levels of CDA polypeptide or CDA-derived
10 polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof in a cell. The change may be an increase or decrease of CDA levels or CDA derived polypeptides in the cell relative to CDA levels in non-cancerous cells or resting cells. Applicants have shown that CDA levels increase in rapidly dividing cancer cells compared to resting cancer cells or
15 normal cells and hence may be used as a cancer marker.

The change in the CDA levels may be a measure of a change in expression of CDA or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants or variants thereof or it may be measured directly as an
20 increase in the amounts of the polypeptide present. Any direct or indirect measurement protocol may be used including the measurement of antibodies to CDA, or binding proteins which bind to CDA.

The cells may be extracted from the patient either as tissue samples or cell
25 cultures may be prepared from the samples. The change in CDA levels may be compared against normal or resting cells, or the change may be measured within the sample as a progressive measurement taken whilst the cells continue to divide.

30 In another aspect of the present invention there is provided a method of increasing cell division, said method including eliminating expression of CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and variants thereof.

The elimination may involve the use of cytotoxic antibodies directed at CDA. The antibodies may be as described above.

5 The present invention will now be more fully described with reference to the accompanying examples and figures. It should be understood, however, that the description following is illustrative only and should not be taken in any way as a restriction of the generality of the invention described.

EXAMPLES

10 **Example 1: Identification of full-length CDA cDNA and mRNA**

A commercial human testis lambda gt11 cDNA library was screened using DLE autoimmune serum. Two overlapping cDNA fragments of CDA (designated hT4 and hT6) were detected. The products of these clones were further confirmed to be immunologically reactive with the autoimmune serum. The nucleic acid
15 sequences of these clones show that they are overlapping clones with identical sequences at the 3' end both containing a poly(A) tail. Based on sequence information from the two clones, a full length clone, hTsl-9, from a testis sub-library was identified. The full length cDNA was found to be 2.8 kb long (Figure 1 or SEQ ID NO:1).

20

The nucleotide sequence of Figure 1 shows the full length cDNA sequence of CDA which is approximately 2.8kb long. The full length CDA nucleotide sequence may include a reading frame that encodes 693 amino acids, preferably from nucleotides 204 to 2284 as represented in Figure 1 or SEQ ID
25 NO:1. The mRNA for CDA has an open reading frame of 2079 bp, encoding predicted polypeptide of 693 amino acids with an apparent molecular weight of 120 kilodaltons and an acidic pI of 4.26. Northern hybridisation on mRNA of HeLa cells and human testis is shown in Figure 6.

30 **Example 2: Determination of molecular weight of CDA.**

GST fusion proteins were made with CDA C-terminal fragments using cDNA clones hT4 and hT6. The proteins were used to immunise rabbits and mice. All animals produced a protein which was reactive with a 120 kDa protein present in a HeLa cell extract. Using two affinity columns to purify the rabbit antibodies

specific to hT4 encoded protein, probing extracts of HeLa, HepG2 and COS cells revealed a 120 kDa protein (Figure 7). In addition, the expressed protein of the CDA1 open reading frame also showed a 120 kDa molecular weight detected by immunoblotting and immunoprecipitation which is shown in the phosphorylation experiment (Figure 5).

Example 3: Transfection of HeLa cells with CDA - Cell cycle effects

Tet-off expression system has been used to establish stable HeLa cell lines over-expressing either full-length or N-terminus CDA. The transgenes could be switched on or off by varying the levels of doxycyclin (Dox) in the medium. Approximately 5000 cells were seeded in 10mm cell culture dishes and cultured with varying concentrations of Dox (5, 0.5, 0.13, 0.06, and 0 ng/mL). After culturing for two weeks, cells were fixed and stained with crystal violet. It was found that cell colony number decreases with lower Dox concentrations (ie transgene switched on) see Figure 8A. This anti-replicative effect was only noted for full-length CDA, and not for the N-terminus only.

As the decreased DOX concentrations result in increased expression levels of CDA transgene, this means that increased CDA level inhibits the out-growth of the cell colonies, hence inhibits the cell growth.

Overexpression of N-terminal truncated form of CDA does not show such inhibitory effect on cell growth indicating the requirement if full length or at least the C-terminus of CDA for its inhibitory function in cell growth.

This dose dependent manner of cell growth inhibition is further investigated by FACS analysis and cell viability studies. Equal numbers of cells were seeded in each well and cultured for 5 days with selected DOX concentrations in the media. Figure 8B shows that when the full length CDA transgene is fully turned on (0.00 ng/ml DOX), all the S phase cells stop DNA synthesising, and fall from R3 to R4, indicating that those cells are arrested. The non-DNA synthesising S phase cells are minimum when the transgene is fully turned off with 5 ng/ml DOX, and increases with decreased DOX concentrations (0.25 ng/ml and 0.03

ng/ml), confirming the dose dependent manner of the effect. Again, this effect was not observed with the N-terminus truncated form of CDA.

Trypan exclusion assays show that the cells that are arrested, the cells are
5 dead. However, the cells with DOX are completely arrested, but over 90% of those cells were still viable meaning a majority of the cells were arrested but not dead.

An experiment of time course expression of CDA shows that the inhibition of
10 increased level of CDA on cell growth takes place 48 hours after the transgene is switched on, and the cells completely stop growing after 72 hours. Figure 9A shows that HeLa cells slow down the growth by cell number measurement 2 days after the full length CDA transgene is turned on compared to the N-terminus. After 3 days and on, the full length cells stop the cell number
15 increasing, while the N-terminus cells are rapidly growing. This confirms the inhibitory effects of CDA on cell growth, and also indicates the requirement of full length or at least the C-terminus to function as an inhibitory protein..

Flow cytometry analysis starts to detect cell cycle arrest on day 3 (Figure 9B).
20 The dot blot shows that S phase cells (R3) are falling down, meaning decreased BrdU incorporation rate. On days 4 and 5, the S phase cells have completely dropped out of R3, meaning that the DNA synthesis fully stops. This event is not observed with the N-terminus cells and is consistent with the findings in the cell growth assay. Trypan blue assays show again, that over 90% of those cells
25 are still viable, meaning that they are arrested rather than dead.

In summary, increased levels of CDA results in cell arrest in all the cell cycle stages.

30 **Example 4: Phosphorylation of CDA**

CDA contains at least two motifs that are potentially phosphorylated by p34^{cdc2} kinase. A mutant CDA was made to have these two potential sites abolished by point mutagenesis. As wild type CDA has the ability to stop the whole cell cycle progression, this mutant fails to show such effect on the cell growth (Figure 10)

indicating that it is essential to phosphorylate CDA, possibly by p34^{cdc2} kinase, to carry out its inhibitory functions in the cell. In addition to the regulation of the CDA levels in various cell cycles, phosphorylation status of CDA is critical, which is possibly regulated by cell cycle kinase, and hence dependent on the cell cycle stages.

Example 5: BrdU incorporation and DNA content in HeLa cells transfected with CDA1-DM (double mutant).

Cells (2×10^5 cells per 8 cm dish) of the stable cell line 2C6D3 expressing Myc-tagged CDA1-DM were cultured for 5 days with doxycycline to keep the transgene turned off (Off) or without doxycycline to turn the transgene on (On). On days 4 and 5, cells were analyzed by flow cytometry for DNA content and BrdU incorporation (Figure 11A) and expression level of myc-tagged CDA1-DM by immunoblotting using anti-myc McAb (Figure 11B). Blots were reprobed with anti- α tubulin McAb to show equal loading of proteins per lane. (Figure 11C): On day 4, cells were harvested for RT-PCR to confirm presence of mutations in transcripts of expressed myc-tagged CDA1-DM transgene. Diagram shows primers for reverse transcription (P-RT) and subsequent PCR. Vector primer (P-Vect) of pTRE (5' -TTTGACCTCCATAGAAGACA- 3') and reverse CDA1 primer P3 (5' -GAGCGTGAAGTATGCGCGG- 3') were used to amplify the fragment containing S20A mutation. CDA1 primers, P2 (5' -CCGCTACTTGACCAATCTGC- 3') and P1 (5' -CACTCTCATTGTTGTCAGTGGT- 3') were used to amplify the fragment containing the T340A mutation. RT-PCR or PCR products derived from (1) cells with transgene turned off, (2) cells with transgene turned on, (3) wild type CDA1 plasmid and (4) mutant CDA1-DM plasmid. 1' - 4': *NarI* Digestion of corresponding PCR products. Lambda DNA *BstEII* digested molecular weight markers used for 1.8% agarose gel electrophoresis.

Example 6: Interacting Proteins of CDA1 in HeLa Cells.

HeLa cell total lysate was separated by SDS-PAGE (top panel: 7.5% gel; bottom panel: 12% gel) and transferred to nitrocellulose membrane. On the blot, an overlay of purified GST fusion protein of CDA1 C-terminus (GST-CDA1-hT4) or GST protein as a control (GST) was incubated at room temperature for 1 hrs.

After wash, the bound GST-CDA1 fusion protein and GST protein were detected using a rabbit anti-GST-hT4 serum. At least 8 HeLa proteins (indicated by arrows in Figure 12) with molecular weights of approximately 15, 30, 53, 60, 70, 90, 100 and 200 kDa were shown to specifically bind to CDA1 C-terminus, which were not shown to bind to GST control protein. Although the identities of these interacting proteins are yet to be known, this finding suggests that CDA1 interacts with multiple proteins that may be involved in a broad range of cellular regulatory pathways related to cell cycle, and perhaps cell proliferation of cancerous cells.

10

The significance of Figure 12 is that identification of the identities of these proteins interacting with CDA1 may reveal 1). mechanisms how CDA1 acts as a cell proliferation inhibitor; 2). reveal some, perhaps novel and cancer unique, proteins/pathways that are involved in cell proliferation regulation. Drugs can be designed to mimic CDA1 actions and interactions with those proteins/pathways for use in cancer treatment

15

20

Finally it is to be understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.

CLAIMS:

1. A nucleotide sequence encoding a cell division autoantigen (CDA) said sequence:
5 having the nucleotide sequence according to Figure 1 or SEQ ID NO:1 or a fragment, functional equivalent , analogue, mutant or variant thereof; or having a sequence capable of hybridizing to the nucleotide sequence of CDA according to Figure 1 or SEQ ID NO:1 or a fragment, functional equivalent, analogue, mutant or variant thereof;
10 having a sequence which is complementary to a sequence according to Figure 1 or SEQ. ID NO:1 or a fragment, functional equivalent , analogue, mutant or variant thereof.
2. A nucleotide sequence which encodes a CDA polypeptide or CDA-
15 derived polypeptide fragment, functional equivalent, analogue, mutant or variant thereof said polypeptide having a polypeptide sequence according to Figure 1 or SEQ ID NO:2. or a fragment, functional equivalent , analogue, mutant or variant thereof
- 20 3. A nucleotide sequence according to claim 1 or 2 including a reading frame encoded by nucleotides 204 to 2284 as represented in Figure 1 or SEQ ID NO:1 or a fragment, functional equivalent , analogue, mutant or variant thereof
- 25 4. A nucleotide sequence according to any one of claims 1 to 3 having nuclear targeting motifs according to Figures 2a or 2b wherein the nuclear targeting motifs are selected from aa 9-15 encoded by nt 153-173; aa 187-203 encoded by nt 687-737; aa 404-407 encoded by nt 1338-1349 and aa 411-414 encoded by nt 1359-1370.
- 30 5. A nucleotide sequence according to any one of claims 1 to 3 having cdc2 kinase phosphorylate sites according to Figures 2a and 2b wherein the cdc2 kinase phosphorylation sites are selected from Ser20 encoded by nt 186-188 and Thr340 encoded by nt 1146-.

6. A nucleotide sequence encoding a fragment of CDA starting from the nucleic acid codon for amino acid 552(aa552) according to Figure 1 or SEQ ID NO:1 or a fragment, functional equivalent, analogue, mutant or variant thereof.
- 5
7. A nucleotide sequence encoding a fragment of CDA starting from the nucleic acid codon for amino acid 432(aa432) according to Figure 1 or SEQ ID NO:1 or a fragment, functional equivalent, analogue, mutant or variant thereof.
- 10
8. An expression vector or cloning vector encoding a CDA said vector including the nucleotide sequence according to anyone of claims 1 to 7.
9. An expression vector according to claim 8 wherein the vector is selected from the group including bacteriophages such as lambda derivatives, or
- 15
- plasmids such as pBR322 or pUC plasmid derivatives.
10. A host cell transformed or transfected with an expression vector or cloning vector according to claim 8 or 9.
- 20
11. A host cell according to claim 10 derived from a host system selected from the group including mammalian cell systems infected with virus; insect cell systems infected with virus; or microorganisms including yeast vectors, or bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA.
- 25
12. A host cell according to claim 11 wherein the bacterium is selected from the group including *E.coli*, *B. Subtilis* or *Salmonella*.
13. An amino acid sequence encoding a CDA and having the amino acid sequence according to Figure 1 or SEQ ID NO:2 or a fragment, functional
- 30
- equivalent, analogue, mutant or variant thereof.
14. An amino acid sequence according to claim 13 wherein the sequence starts from amino acid 552.

15. An amino acid sequence according to claim 13 wherein the sequence starts from amino acid 432.
16. An isolated or substantially pure CDA polypeptide encoded by a
5 nucleotide sequence according to anyone of claims 1 to 7.
17. An isolated or substantially pure CDA polypeptide encoded by an amino acid sequence according to anyone of claims 13 to 15.
- 10 18. A polypeptide according to claim 16 or 17 having an apparent molecular weight of approximately 120kDa.
19. A polypeptide according to anyone of claims 16 to 18 isolated from sera, cells or tissue.
- 15 20. A polypeptide according to claim 19, wherein the sera is obtained from patients having discoid lupus erythematosus (DLE).
21. A polypeptide according to claim 19 or 20 wherein the cells are rapidly
20 dividing cells selected from the group including HeLa cells, HepG2 cell lines or COS cells derived from the African Green Monkey.
22. A polypeptide according to anyone of claims 16 to 21 which is phosphorylated.
- 25 23. A polypeptide according to claim 22 wherein a serine residue or a threonine residue is phosphorylated.
24. A polypeptide according to claim 23 wherein Ser20 (aa 20 or S20) or Thr
30 340 (aa 340 or T340) according to Figure 1 or SEQ ID NO:2 is phosphorylated.
25. An antibody that specifically binds CDA polypeptide or CDA-derived polypeptides, fragments, functional equivalents, analogues, mutants, and

variants thereof, wherein said polypeptide is according to any one of claims 16 to 24.

26. A vaccine comprising a CDA polypeptide according to any one of claims
5 16 to 24.

27. A method of controlling cell division, said method including subjecting
cells to an effective amount of a CDA or CDA-derived polypeptide, fragment,
functional equivalent, analogue, mutant, or variant thereof.
10

28. A method according to claim 27 wherein the CDA is according to any one
of claims 16 to 24.

29. A method according to claim 27 for reducing cell division, said method
15 comprising inducing expression and/or activity of CDA or a CDA-derived
polypeptide, fragment, functional equivalent, analogue, mutant, or variant
thereof.

30. A method of controlling cell division, said method including subjecting
20 cells to an effective amount of an antagonist of CDA or CDA-derived
polypeptide, fragment, functional equivalent, analogue, mutant, or variant
thereof.

31. A method according to claim 30 wherein the antagonist is an antibody or
25 peptide directed to the CDA polypeptide or binding site of the CDA.

32. A method according to claim 30 or 31 wherein the antagonist prevents
phosphorylation of the CDA.

30 33. A method according to claim 30 for increasing cell division, said method
including reducing expression and/or activity of a CDA polypeptide or CDA-
derived polypeptide, fragment, functional equivalent, analogue, mutant, or
variant thereof.

34. A method according to claim 33 wherein the CDA is according to anyone of claims 16 to 24.

5 35. A method of preventing and treating cancer, said method including providing an effective amount of a CDA or CDA-derived polypeptide, fragment, functional equivalent, analogue, mutant, or variant thereof to a patient in need, said effective amount being effective to halt or slow down cell growth.

10 36. A method according to claim 35 wherein the CDA is according to anyone of claims 16 to 24.

15 37. A method according to claim 35 or 36 wherein the CDA is provided by inducing expression and/or activity of a CDA or CDA-derived polypeptide, fragment, functional equivalent, analogue, mutant, or variant thereof in a cell.

38. A method of detection of cell cycle activity, said method including measuring the presence of a CDA polypeptide or CDA-derived polypeptide, fragment, functional equivalent, analogue, mutant, or variant thereof.

20 39. A method according to claim 38 wherein the CDA is according to anyone of claims 16 to 24.

25 40. A method according to claim 38 or 39 wherein the detection of cell cycle activity is a measure of expression of CDA or a CDA-derived polypeptide, fragment, functional equivalent, analogue, mutant, or variant thereof.

30 41. A method of diagnosis of cancer, said method comprising determining a change in levels of CDA polypeptide or CDA-derived polypeptide, fragment, functional equivalent, analogue, mutant, or variant thereof in a cell.

42. A method according to claim 41 wherein the change is a measure of expression of CDA or a CDA-derived polypeptide, fragment, functional equivalent, analogue, mutant, or variant thereof.

43. A method according to claim 42 wherein the expression is measured by a change in transcription of the CDA polypeptide.

44. A method according to claim 41 wherein the change is measured by
5 antibodies to CDA or CDA-derived polypeptide, fragment, functional equivalent, analogue, mutant, or variant thereof.

45. A method according to claim 41 wherein an increase in CDA levels is an indication of cancer.

1/17

1 CGAGGTGGTGAGGAGAGCTGGTTGCGTGAGTCTCCTCAGCTCTGCTTACCGGT 53
 54 GCGACTAGCGGCAGCGACGCGGGCTAAAAGCGAAGGGGCGAGTGCAGTCCCTGAGCTGTACGAACGCGGTGCGC 128
 129 ATGGACCGCCAGATGAGGGGCTCCGGCCAAGACCCGCGCTGAGCAGTCCGAGTCTCCACAGCGCGACCCG 203
 1 M D R P D E G P P A K T R R L S S S E S P Q R D P 25
 204 CCCCCGCGCGCCGCGCCGCGCGCTCCTCCGACTGCCGCTGCCTCCACCCAGCAGCGCCCGAGGCTCCAGGAG 278
 26 P P P P P P P P L L R L P L P P P Q Q R P R L Q E 50
 279 GAAACGGAGGCGGCACAGGTGCTGGCCGATATGAGGGGGTGGGACTGGGCCCGCGCTGCCCGCGCGCTCCC 353
 51 E T E A A Q V L A D M R G V G L G P A L P P P P P 75
 354 TATGTCTATCTCGAGGAGGGGGGATCCGCGCATACTTCACGCTCGGTGCTGAGTGTCCCGGTGGGATTCTACC 428
 76 Y V I L E E G G I R A Y F T L G A E C P G W D S T 100
 429 ATCGAGTCGGGGTATGGGGAGGCGCCCCCGACGGAGGCTGGAAGCACTCCCCACTCTGAGGCCTCGGGG 503
 101 I E S G Y G E A P P P T E S L E A L P T P E A S G 125
 504 GGGAGCCTGGAATCGATTTCAGGTTGTACAGTCGAGCAGTTTGGTGGAGAGGGGGCCCTAGAAACCTGTAGC 578
 126 G S L E I D F Q V V Q S S S F G G E G A L E T C S 150
 579 GCAGTGGGGTGGGCGCCCCAGAGGTTAGTTGACCCGAAGAGCAAGGAAGAGGCGATCATCATAGTGAGGATGAG 653
 151 A V G W A P Q R L V D P K S K E E A I I I V E D E 175
 654 GATGAGGATGAGCGGGAGAGTATGAGGAGCAGCAGGAGCGCGCGCGGAGGAGGAGCAAGAGGAGGATG 728
 176 D E D E R E S M R S S R R R R R R R R R R K Q R K V 200
 729 AAGAGGGAAGCAGAGAGAGAAATGCCGAGAGGATGGAGAGCATCTGCAGGCACTGGAGGATATTCAGCTGGAT 803
 201 K R E S R E R N A E R M E S I L Q A L E D I Q L D 225
 804 CTGGAGGCAGTGAACATCAAGGCAGGCAAGCCTTCTGCGTCTCAAGCGCAAGTTCATCCAGATGCGAAGACCC 878
 226 L E A V N I K A G K A L K R K F I R K R P 250
 879 TTCCTGGAGCGCAGAGACCTCATCATCCAGCATATCCAGGCTTCTGGGTCAAAGCATTCTCAACCAACCCAG 953
 251 F L E R R D L I I Q H I P G F W V K A F L N H P R 275
 954 ATTTCAATTTTGATCAACCGACGTGATGAAGACATTTCCGCTACTTGACCAATCTGCAGGTACAGGATCTCAGA 1028
 276 I S I L I N R R D E D I F R Y L T N L Q V Q D L R 300
 1029 CATATCTCCATGGGCTACAAATGAAGCTTACTTCCAGACTAACCCCTACTTCAAAACATGCTGATTGTCAAG 1103
 301 H I S M G Y K M K L Y F Q T N P Y F T N M V I V K 325
 1104 GAGTTCAGCGCAACCGCTCAGGCCGGCTGGTGTCTCACTCAACCCCAATCCGCTGGCACCGGGGCCAGGAACCC 1178
 326 E F Q R N R S G R L V S H S T P I R W H R G Q E P 350
 1179 CAGGCCCGTCTGTCACGGGAACAGGATGCGAGCCACAGCTTTTTCAGCTGGTTCTCAAACCATAGCCTCCAGAG 1253
 351 Q A H G N Q D A S H S F F S N H S N H S P E 375
 1254 GCTGACAGGATTGCTGAGATTATCAAGAATGATCTGTGGGTTAACCTCTACGCTACTACCTGAGAGAAAGGGGC 1328
 376 A D R I A E I I K N D L W V N P L R Y Y L R E R G 400
 1329 TCCAGGATAAAGAGAAAGAAGCAAGAAATGAAGAAACGTAACCAACAGGGGCGAGATGTGAGGTGGTGATCATGGAA 1403
 401 S R I K R K K Q E M K K R K T R G R C E V V I M E 425
 1404 GACGCCCCCTGACTATTATGCACTGGAAGACATTTTCAGCGAGATCTCAGACATTGATGAGACAAATTCATGACATC 1478
 426 D A P D Y Y A V E D I F S E I S D I D E T I H D I 450
 1479 AAGATCTCTGACTTCATGGAGACCACCGACTACTTCGAGACCACTGACAATGAGATAACTGACATCAATGAGAAC 1553
 451 K I S D F M E T T D Y F E T T D N E I T D I N E N 475
 1554 ATCTGCGCAGCGAGAATCCTGACCACAATGAGGTCCCCAACACGAGACCACTGATAACAACGAGAGTGTGAT 1628
 476 I C D S E N P D H N E V P N E T T D N N E S A D 500
 1629 GACCACGAAACCACTGACAACAATGAGAGTGCAGATGACAACAACGAGAATCCTGAAGACAATAACAAGAACT 1703
 501 D H E T T D N N E S A D D N N E N P E D N N K N T 525
 1704 GATGACAACGAAGAGAACCCTAACAAACAGAGAACACTTACGGCAACAACCTTCTCAAAGGTGGCTTCTGGGGC 1778
 526 D D N E N P N N E N E N T Y G N N F F K G F W G 550
 1779 AGCCATGGCAACCAACAGGACAGCAGCATGACAATGAAGCAGATGAGGCCAGTGAATGATGATAAGATAATGAT 1853
 551 S H G N N Q D S S D S D N E A D E A S D D E D N D 575
 1854 GGCAACGAAGGTGACAATGAGGGCAGTGATGATGATGGCAATGAAGGTGACAATGAAGGCAGCGATGATGACGAC 1928
 576 G N E G D N E G S D D D G N E G D N E G S D D D D 600
 1929 AGAGACATTGAGTACTATGAGAAAGTTATGAAGACTTTGACAAGGATCAGGCTGACTACGAGGACGTGATAGAG 2003
 601 R D I E Y Y E K V I E D F D K D Q A D Y E D V I E 625
 2004 ATCATCTCAGACGAATCAGTGAAGAAGAGGGCATTGAGGAAGGCATCCAGCAAGATGAGGACATCTATGAGGAA 2078
 626 I I S D E S V E E E G I E E G I Q Q D E D I Y E E 650
 2079 GGAACCTATGAGGAGGAAGGAAGTGAAGATGTCTGGGAAGAAGGGGAAGATTCGGACGACTCTGACCTAGAGGAT 2153
 651 G N Y E E E G S E D V W E E G E D S D D S D L E D 675
 2154 GTGCTTCAGGTCCCAACGGTTGGGCCAATCCGGGGGAAGAGGGGGAAACCGGATAAGGGTTTTCCCTTTGGGG 2228
 676 V L Q V P N G W A N P G K R G K T G • 694
 2229 GATCACCTCTCTGTATCCCCACCCACTATCCCATTTGCCCTCCTCCTCAGTAGGGCCACGCGGCCCCACATTG 2303
 2304 CACTTCTGGGGGGTGACCGACTTCGTACACGGGTTTAAAGTTTATTTTATGGTTTATGTCAGAGTTCTTA 2378
 2379 TTTTGGGGGGAGGGAAAGGGGGCTAGTCCCCCTTCTTTGGCCCTCCGCCCCCGCAGGCTTCTGTGTGTGCTAAC 2453
 2454 TGTATTATTGTGATGCCTTGGTCAGGGCCCTTACCACCTTCTCCAGTCAGTTGTGGCCCCAGCCCTCTCC 2528
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 2604 CCCATGGAGGCGCTGCTGCCACCTTCTCTCCCAAGTTCTTCTCCATCCCTCTCCTCTTCCCGCCGCGCGCTA 2678
 2679 GCCCGCTCGGTGTCTATGCAAGGCCCTTCGCCATTGCGGTATTCTTTGCGGTATTCTTGTCCCCGTCCCCAGA 2753
 2754 AGGCTCGCCTCTCCCCGTGGACCTGTAAATCCCAATAAAATCTGAGCAAGTTCAAAAAAAAAAAAAAAAAAAAA 2828
 2829 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

FIG 1

SUBSTITUTE SHEET (RULE 26)

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1 CGAGGTGGTGAGGAGAGCTGGTTGCGTGAGTCTCCTCAGCTCTGCTTACCGGT 53
54 GCGACTAGCGGCAGCGACGCGGCTAAAAGCGAAGGGGCGAGTGCGAGTCCCCTGAGCTGTACGAACGCGGTCCGC 128
129 ATGGACCGCCAGATGAGGGGCTCCGGCCAAGACCCGCCGCTGAGCAGCTCCGAGTCTCCACAGCGCGACCCG 203
1 M D R P D E G P P A K T R R L S S S D S P D P 25
204 CCCCCGCGCCGCGCCGCGCGCTCCTCCGACTGCCCTGCCCTCCACCCAGCAGCGCCGAGGCTCCAGGAG 278
26 P P P P P P P L L R L P L P P P Q Q R P R L Q E 50
279 GAAACGGAGGCGGCACAGGTGCTGGCCGATATGAGGGGGTGGGACTGGGCCCCGCGCTGCCCCCGCCCTCCC 353
51 E T E A A Q V L A D M R G V G L G P A L P P P P P 75
354 TATGTCATTCTCGAGGAGGGGGGATCCGCGCATACTTACGCTCGGTGCTGAGTGTCCGGCTGGGATTCTACC 428
76 Y V I L E E G G I R A Y F T L G A E C P G W D S T 100
429 ATCGAGTCGGGGTATGGGGAGGCGCCCCCGCCACGGAGAGCCTGGAAGCACTCCCCACTCTGAGGCCTCGGGG 503
101 I E S G Y G E A P P P T E S L E A L P T P E A S G 125
504 GGGAGCCTGGAATCGATTTTCAGGTTGTACAGTCGAGCAGTTTTGGTGAGAGGGGGCCCTAGAAACCTGTAGC 578
126 G S L E I D F Q V V Q S S S F G G E G A L E T C S 150
579 GCAGTGGGGTGGGCGCCCCAGAGGTTAGTTGACCCGAAGAGCAAGGAAGAGGCGATCATCATAGTGGAGGATGAG 653
151 A V G W A P Q R L V D P K S K E E A I I I V E D E 175
654 GATGAGGATGAGCGGGAGAGTATGAGGAGCAGCAGGAGGCGGCGGCGGCGGAGGAGGAAGCAGAGGAAGGTG 728
176 D E D E R E S M R S S R R R R R R R R R R K Q R K V 200
729 AAGAGGAAAGCAGAGAGAGAAATGCCGAGAGGATGGAGAGCATCTGCAGGCACTGGAGGATATTCAGCTGGAT 803
201 K R E S R E R N A E R M E S I L Q A L E D I Q L D 225
804 CTGGAGGCAGTGAACATCAAGGCAGGCAAAGCCTTCTGCGTCTCAAGCGCAAGTTCATCCAGATGCGAAGACCC 878
226 L E A V N I K A G K A F L R L K R K F I Q M R R P 250
879 TTCCTGGAGCGCAGAGACCTCATCATCCAGCATATCCAGGCTTCTGGGTCAAAGCATTCCTCAACCACCCAGA 953
251 F L E R R D L I I Q H I P G F W V K A F L N H P R 275
954 ATTTCAATTTTGATCAACCGACGTGATGAAGACATTTTCCGCTACTTGACCAATCTGCAGGTACAGGATCTCAGA 1028
276 I S I L I N R R D E D I F R Y L T N L Q V Q D L R 300
1029 CATATCTCCATGGGCTACAAAATGAAGCTGTACTTCCAGACTAACCCCTACTTCACAAACATGGTGATTGTCAAG 1103
301 H I S M G Y K M K L Y F Q T N P Y F T N M V I V K 325
1104 GAGTTCAGCGCAACCGCTCAGGCCGGCTGGTGTCTACTCAACCCCAATCCGCTGGCACCAGGGGCCAGGAACCC 1178
326 E F Q R N R S G R L V S H S P L L W H R G Q E P 350
1179 CAGGCCCGTCGTCACGGGAACCAGGATGCGAGCCACAGCTTTTTCAGCTGGTCTCAAACCATAGCCTCCAGAG 1253
351 Q A R R H G N Q D A S H S F F S W F S N H S L P E 375
1254 GCTGACAGGATTGCTGAGATTATCAAGAATGATCTGTGGGTTAACCTCTACGCTACTACCTGAGAGAAAGGGC 1328
376 A D R I A E I I K N D L W V N P L R Y Y L R E R G 400
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401 S R I K R K K Q E M K K R K T R G R C E V V I M E 425
1404 GACGCCCTGACTATTATGCAGTGGAAACATTTTCAGCGAGATCTCAGACATTGATGAGACAATTCATGACATC 1478
426 D A P D Y Y A V E D I F S E I S D I D E T I H D I 450
1479 AAGATCTCTGACTTCATGGAGACCACCGACTACTTCGAGACCACTGACAATGAGATAACTGACATCAATGAGAAC 1553
451 K I S D F M E T T D Y F E T T D N E I T D I N E N 475
1554 ATCTGCGACAGCGAGAATCCTGACCACAATGAGGTCCCCAACGAGACCACTGATAACAACGAGAGTGCTGAT 1628
476 I C D S E N P D H N E V P N N E T T D N N E S A D 500
1629 GACCACGAAACCACTGACAACAATGAGAGTGAGATGACAACAACGAGAATCCTGAAGACAATAACAAGAACACT 1703
501 D H E T T D N N E S A D D N N E N P E D N N K N T 525
1704 GATGACAACGAAGAGAACCCCTAACAAACGAGAACACTTACGGCAACAACTTCTTCAAAGGTGGCTTCTGGGGC 1778

FIG 2a

SUBSTITUTE SHEET (RULE 26)

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526 D D N E E N P N N N E N T Y G N N F F K G G F W G 550
1779 AGCCATGGCAACAACCAGGACAGCAGCGACAGTGACAATGAAGCAGATGAGGCCAGTGATGATGAAGATAATGAT 1853
551 S H G N N Q D S S D S D N E A D E A S D D E D N D 575
1854 GGCAACGAAGGTGACAATGAGGGCAGTGATGATGATGGCAATGAAGGTGACAATGAAGGCAGCGATGATGACGAC 1928
576 G N E G D N E G S D D D G N E G D N E G S D D D D 600
1929 AGAGACATTGAGTACTATGAGAAAGTTATTGAAGACTTTGACAAGGATCAGGCTGACTACGAGGACGTGATAGAG 2003
601 R D I E Y Y E K V I E D F D K D Q A D Y E D V I E 625
2004 ATCATCTCAGACGAATCAGTGGAAGAAGAGGGCATTGAGGAAGGCATCCAGCAAGATGAGGACATCTATGAGGAA 2078
626 I I S D E S V E E E G I E E G I Q Q D E D I Y E E 650
2079 GGAAACTATGAGGAGGAAGGAAGTGAAGATGTCCTGGGAAGAAGGGGAAGATTCGGACGACTCTGACCTAGAGGAT 2153
651 G N Y E E E G S E D V W E E G E D S D D S D L E D 675
2154 GTGCTTCAGTCCCAAACGGTTGGGCCAATCCGGGGAAGAGGGGGAACCCGGATAAGGGTTTTCCCTTTGGGG 2228
676 V L Q V P N G W A N P G K R G K T G • 694
2229 GATCACCTCTCTGTATCCCCACCCACTATCCCATTTGCCCTCCTCCTCAGCTAGGGCCACGCGCCCCACATTG 2303
2304 CACTTCTGGGGGGTGACCGACTTCGTACACGGGTTTAAAGTTTATTTTATGGTTTAGTCATTGCAGAGTTCTTA 2378
2379 TTTTGGGGGGAGGGAAAGGGGGCTAGTCCCCCTTCTTTTGGCCCTCCGCCCCGCAGGCTTCTGTGTGCTGCTAAC 2453
2454 TGTATTTATTTGTGATGCC'TTGGTCAGGGCCCCCTCTACCCACTTCTCCCAGTCAGTTGTGGCCCCAGCCCCCTCTCC 2528
2529 CTGTGCTGTGTGGAGTGGACACCCTGACCCCCGAAGCGGGGAGGGCCGCTGTGGCCTTCGTACAGCCGCGCAGT 2603
2604 CCCATGGAGGCGCTGCTGCCACCTTCTCTCCCAAGTTCTTTCTCCATCCCTCTCCTCTTCCCGCCGCGCCGCTA 2678
2679 GCCCGCCTCGGTGTCTATGCAAGGCCCTTCGCCATTGCGGTATTCTTTGCGGTATTCTTGTCCCCGTCCCCCAGA 2753
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2829 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 2865

FIG 2b

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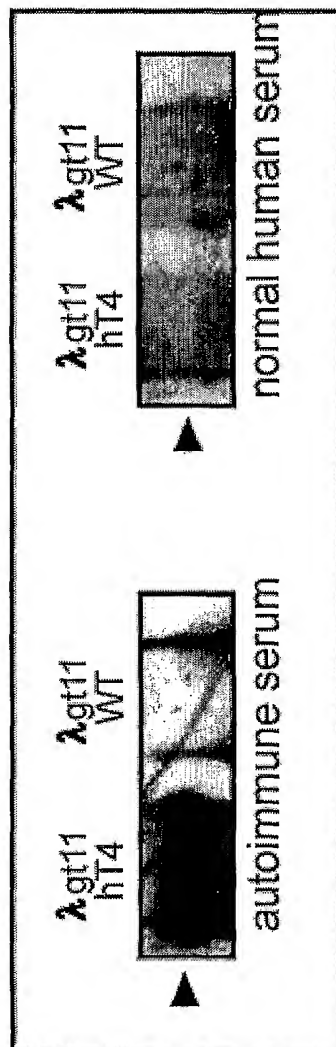


FIG 3

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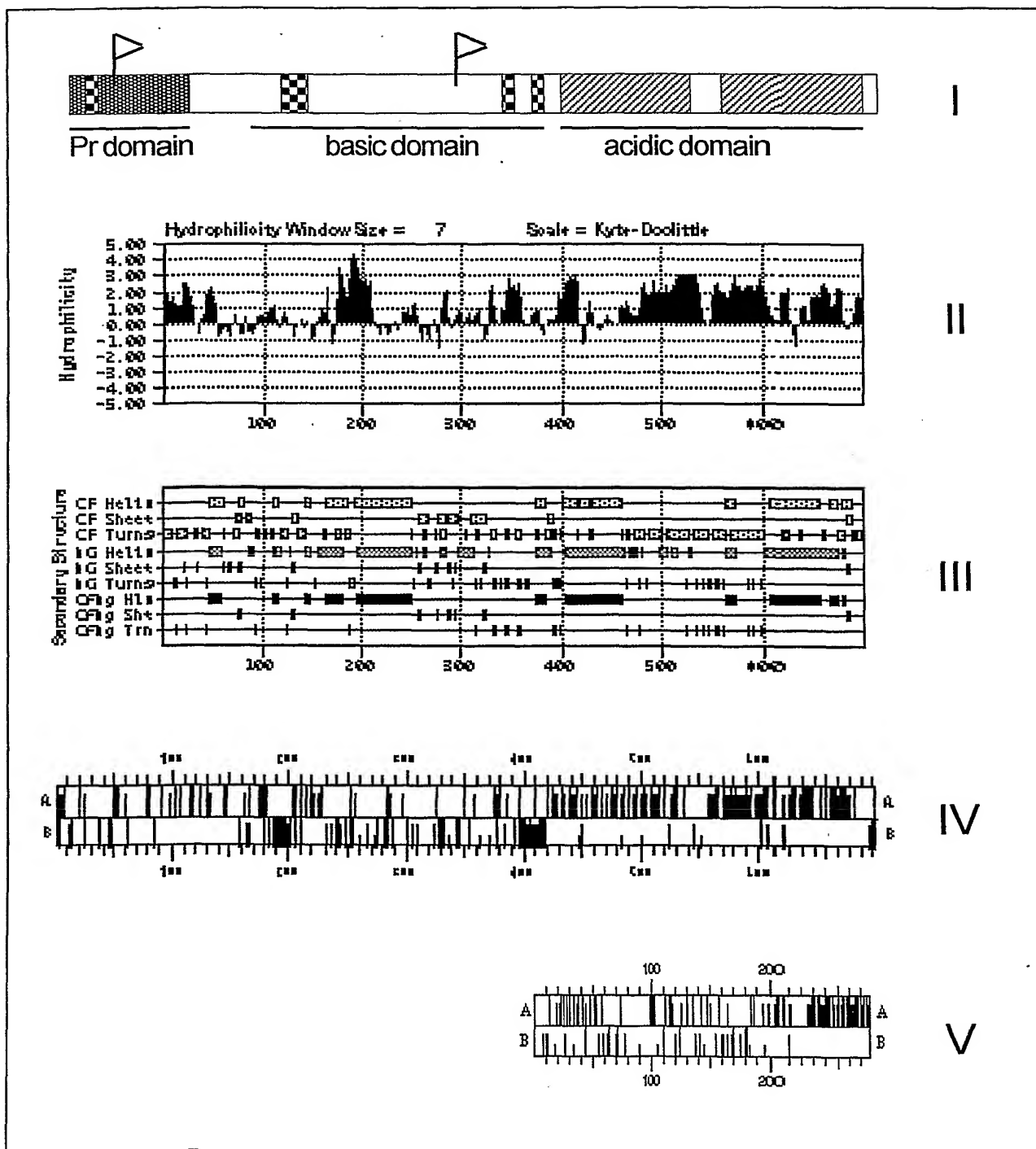
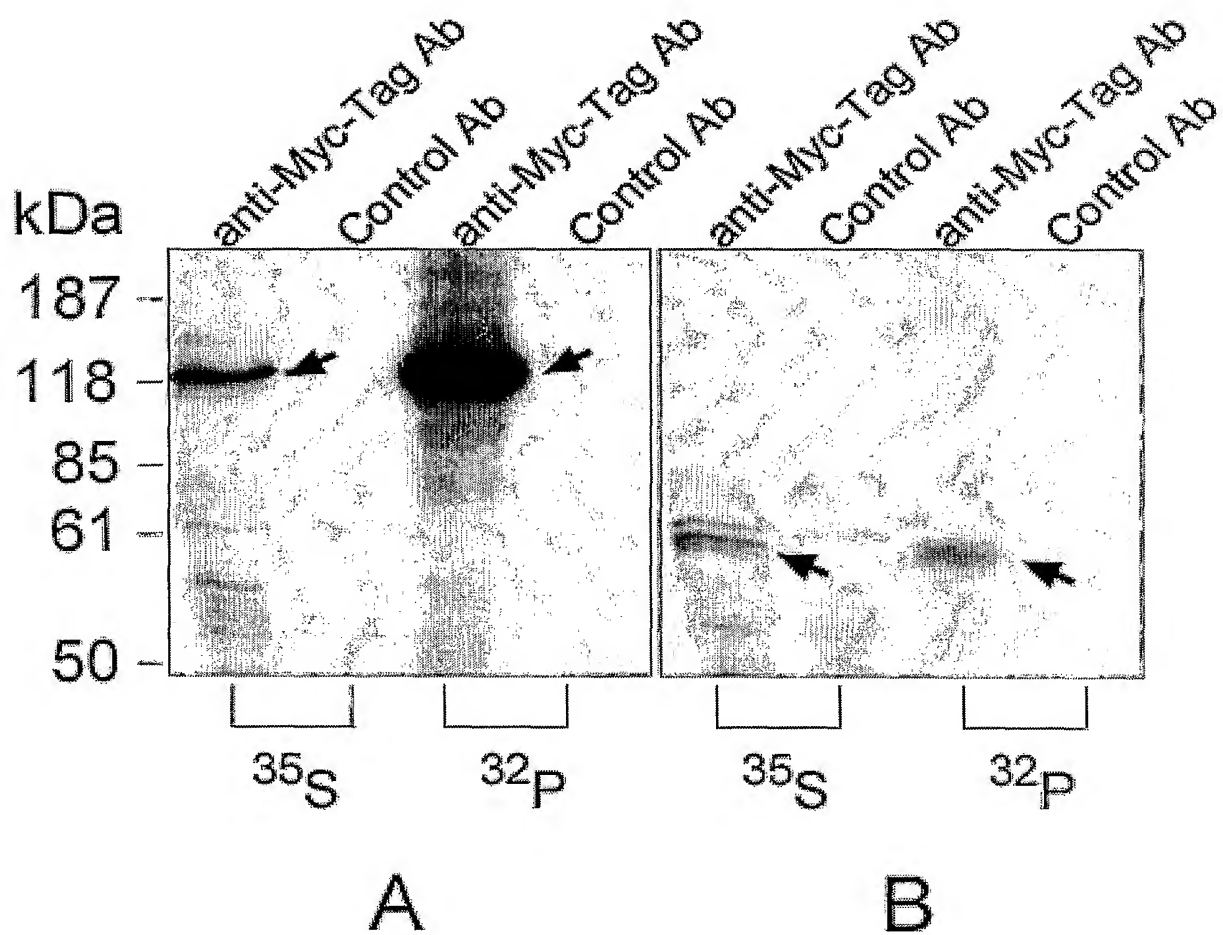


FIG 4

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**FIG 5**

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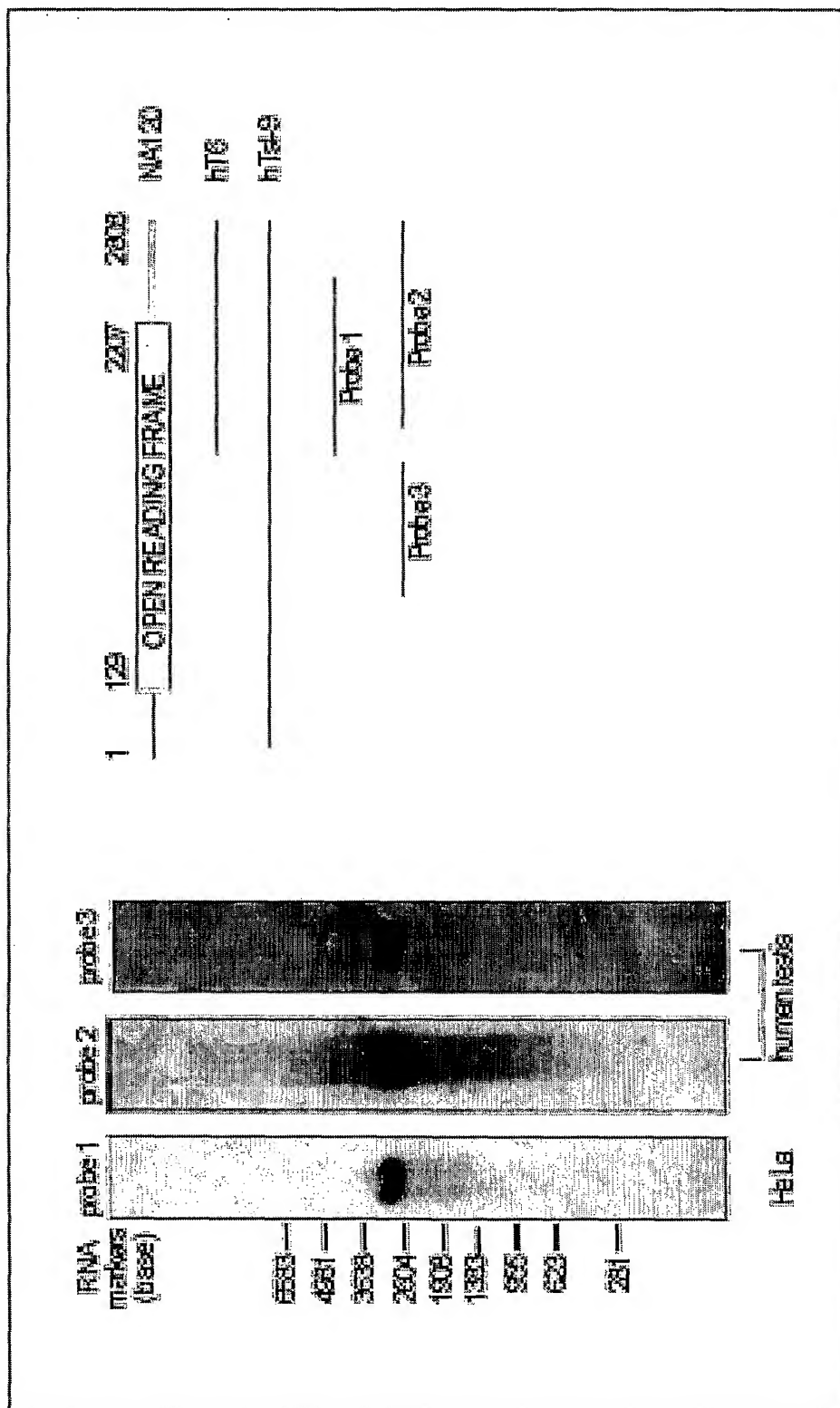


FIG 6

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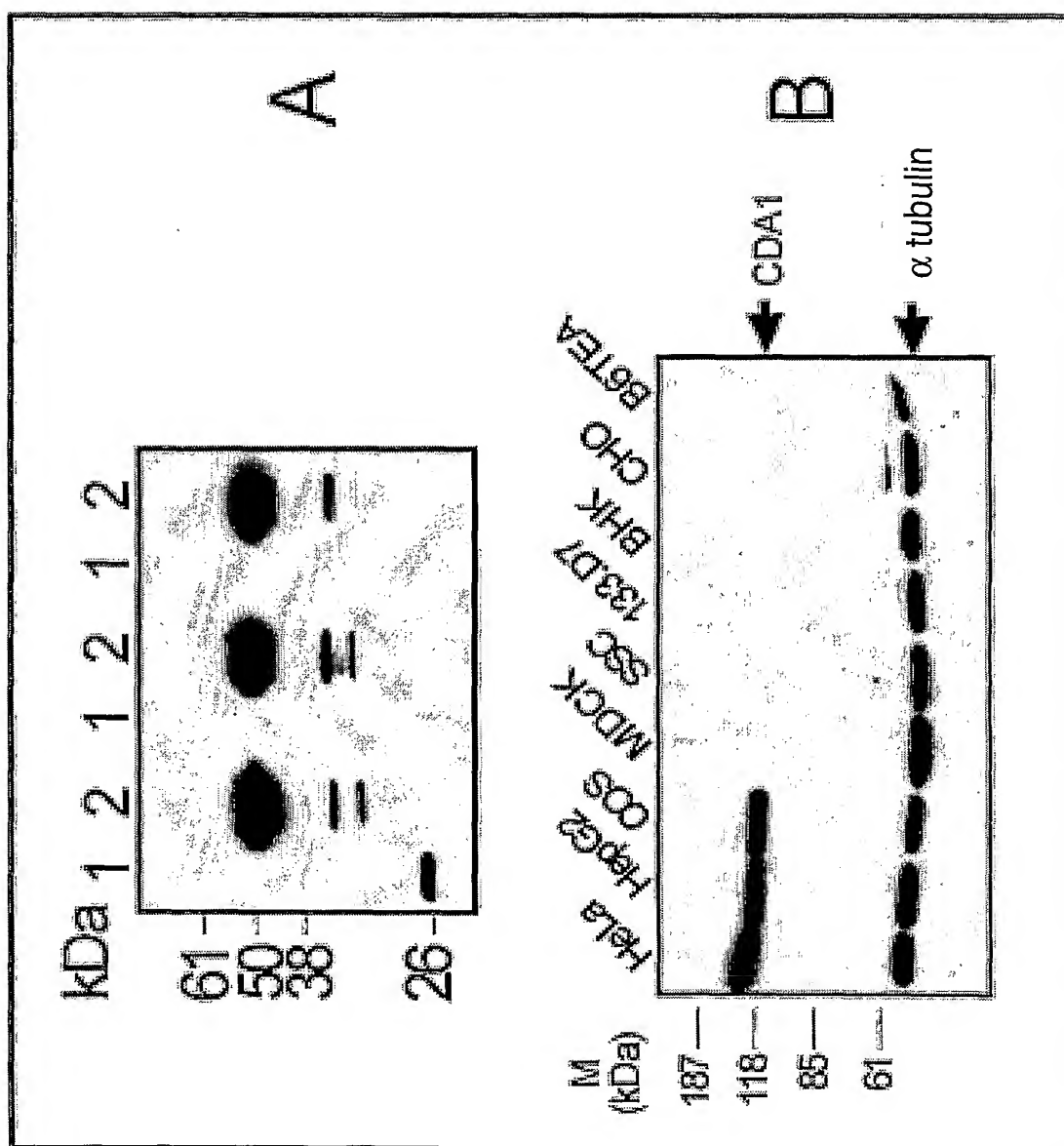
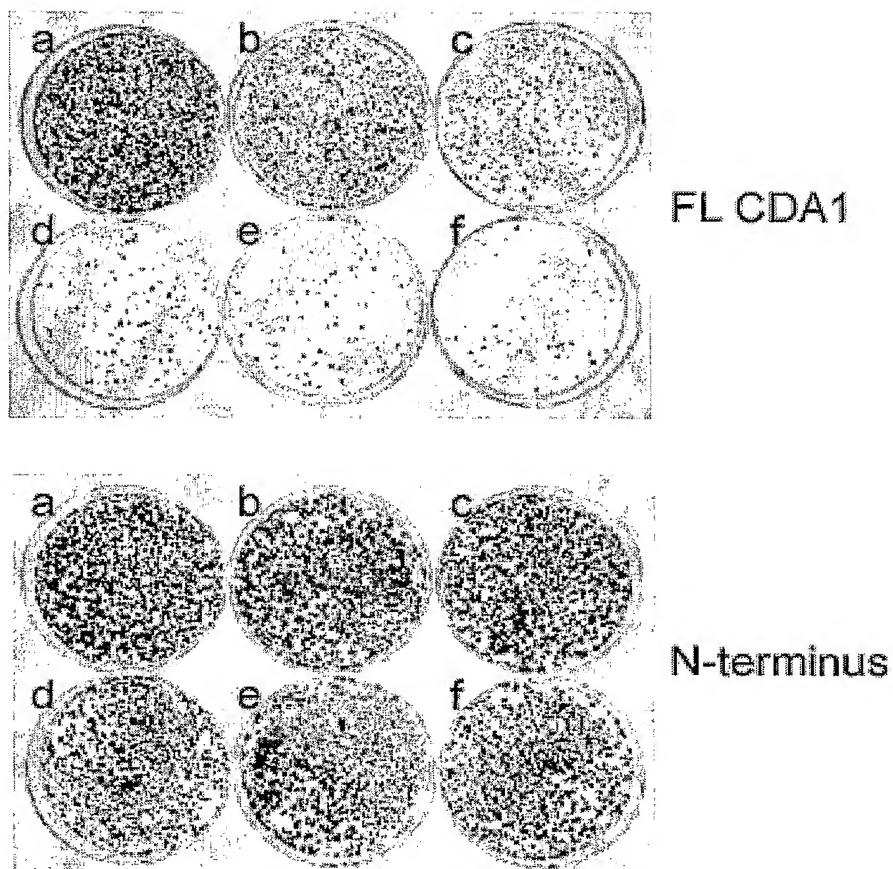


FIG 7

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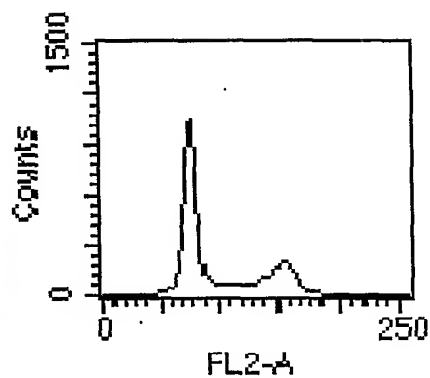
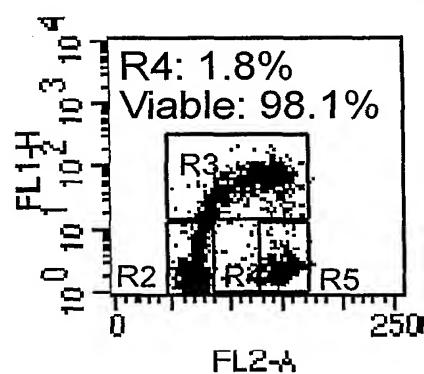


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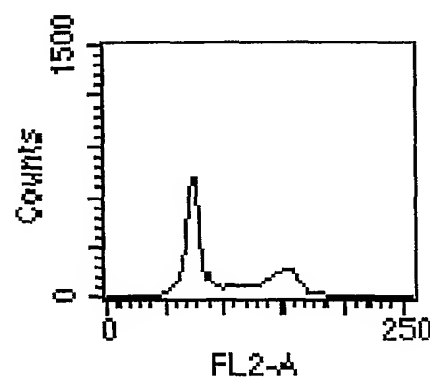
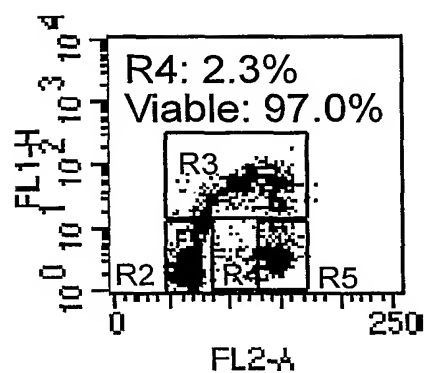
FIG 8a

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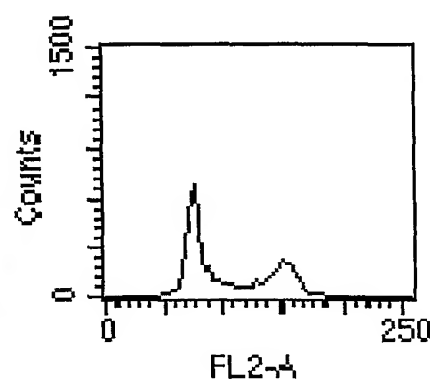
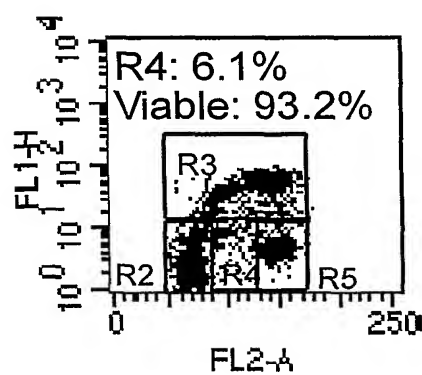
Full Length CDA1

[DOX]
(ng/mL)

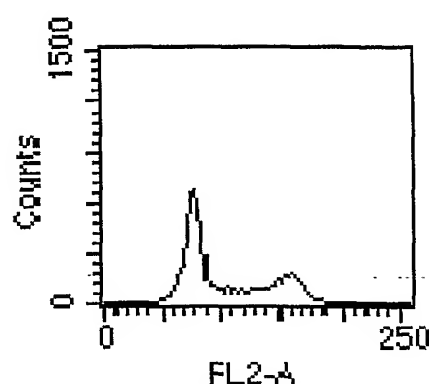
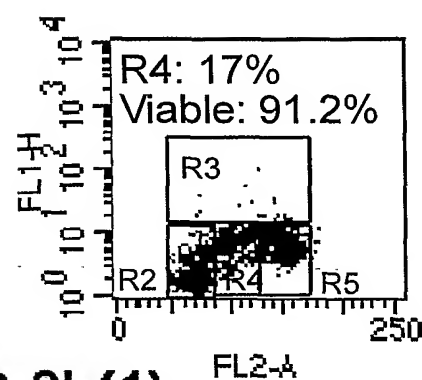
5.00



0.25



0.03



0.00

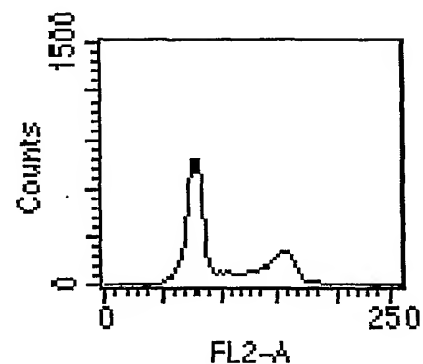
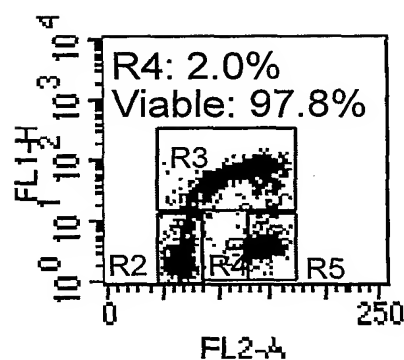
FIG 8b(1)

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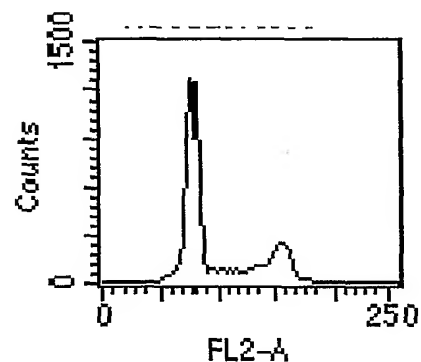
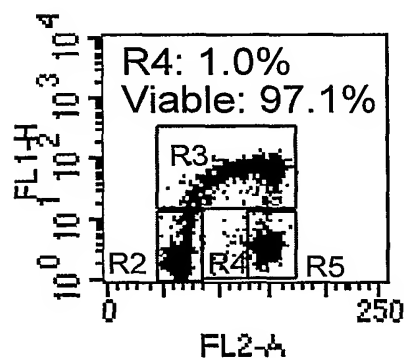
N-terminus of CDA1

[DOX]
(ng/mL)

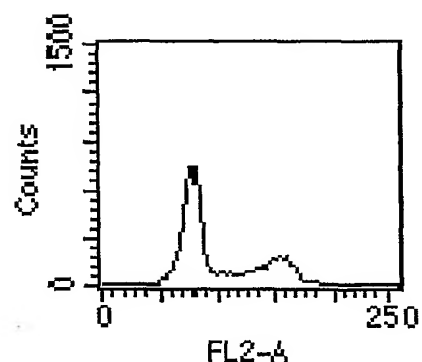
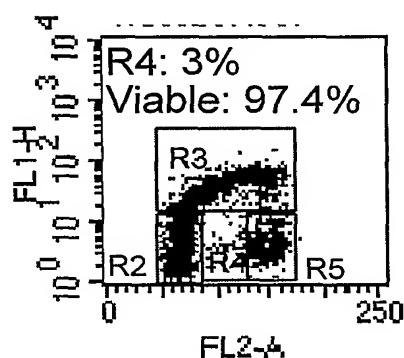
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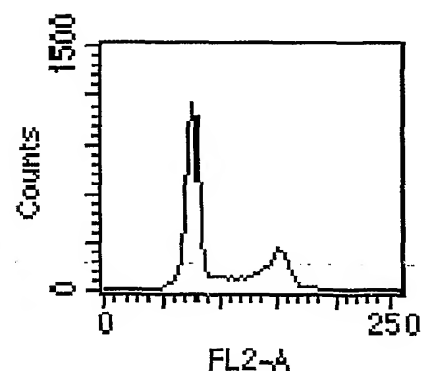
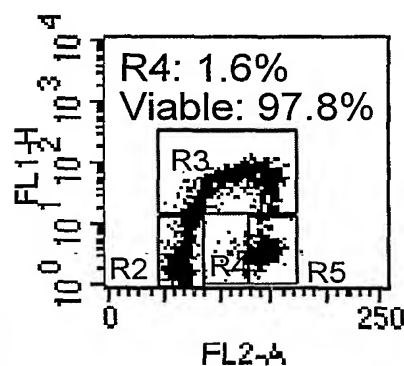


FIG 8b(2)

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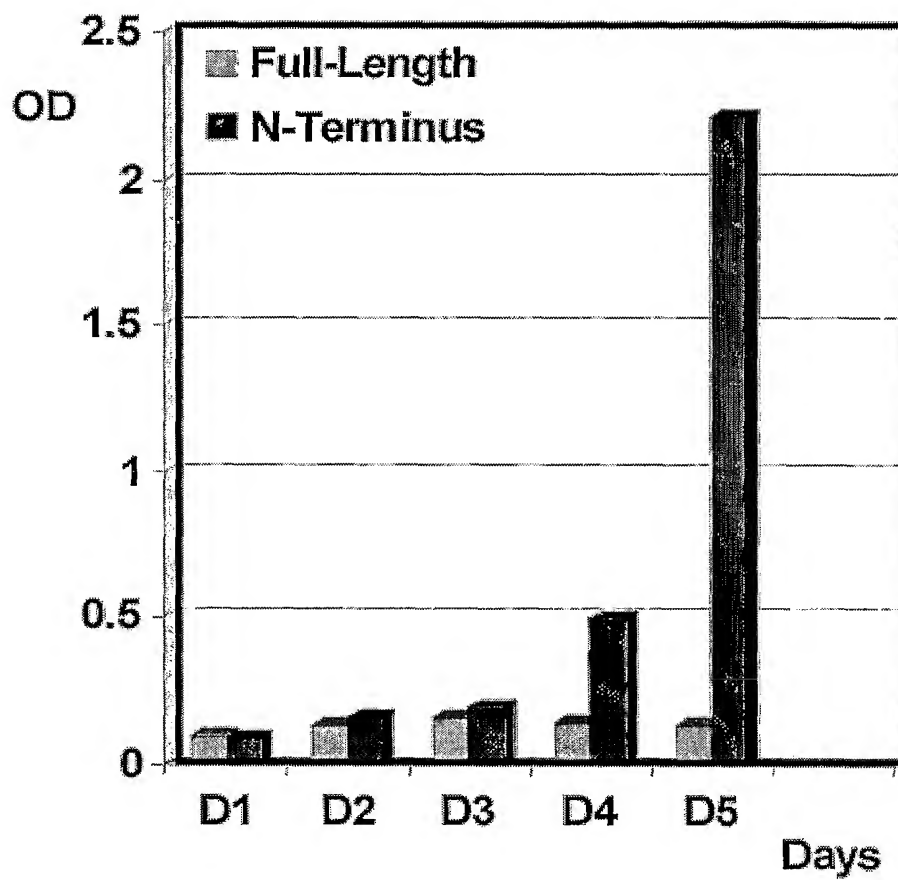


FIG 9a

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Full Length CDA1

Days

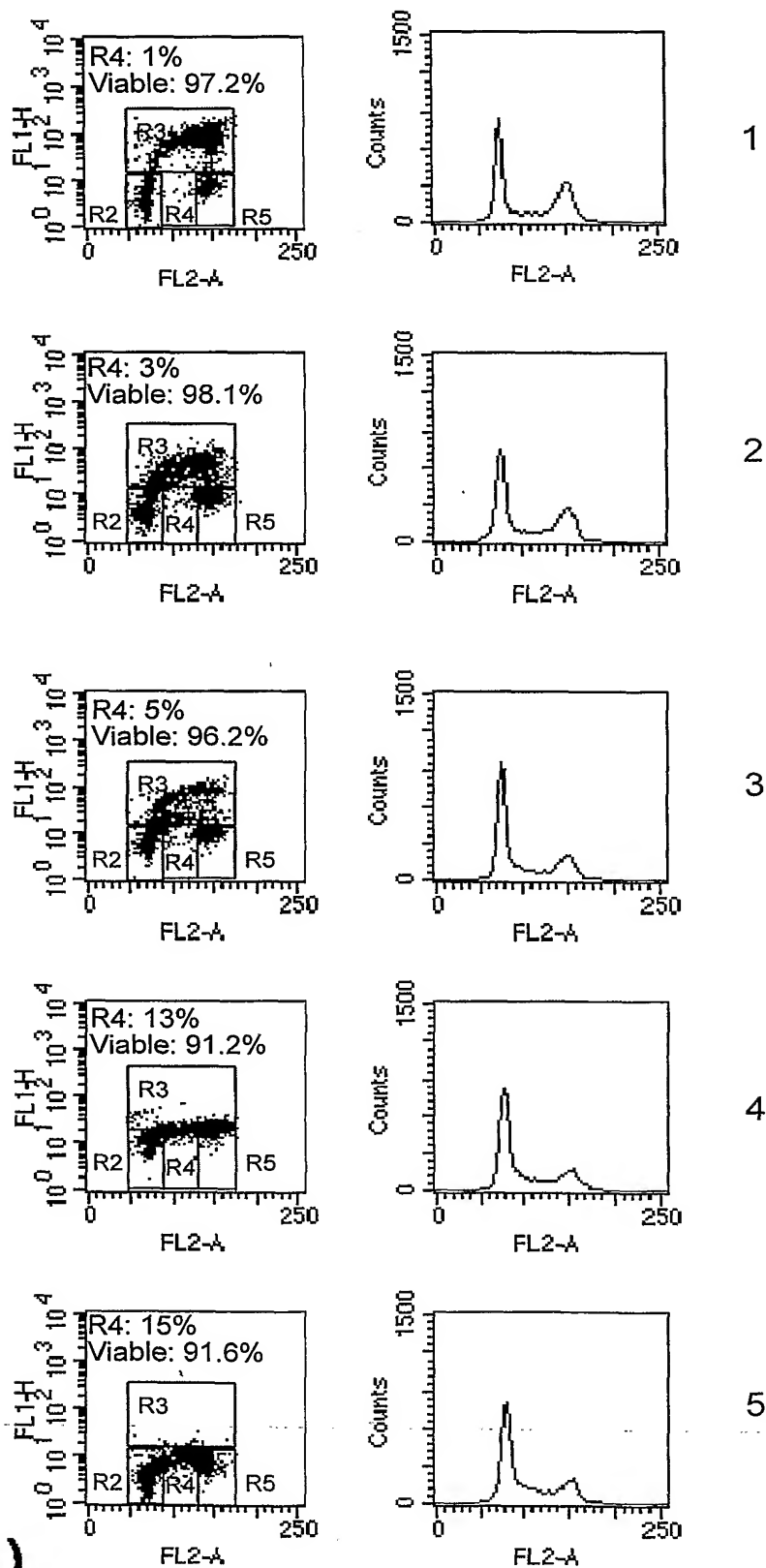


FIG 9b(1)

Days 14/17 N-terminus of CDA1

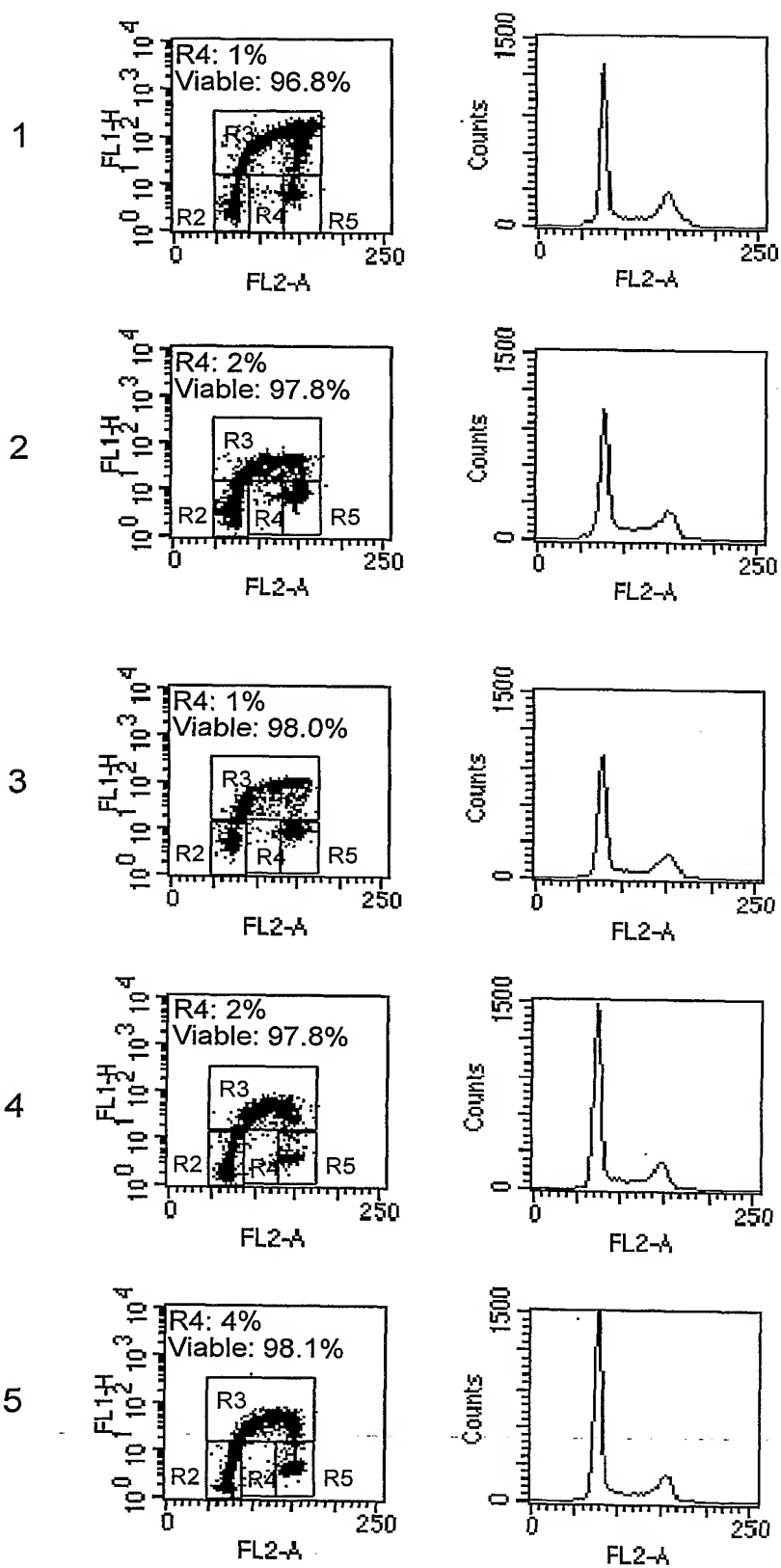


FIG 9b(2)

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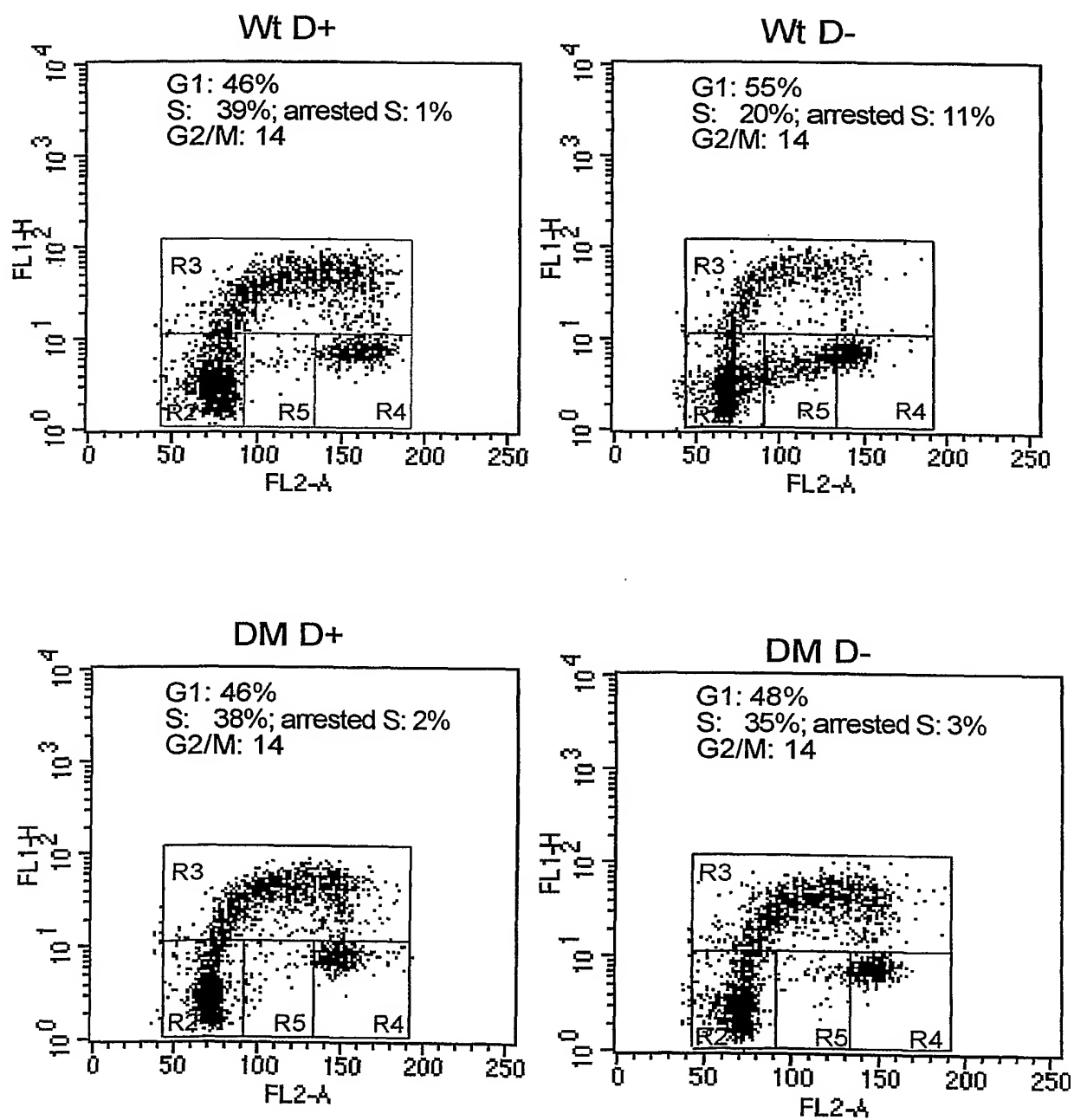


FIG 10

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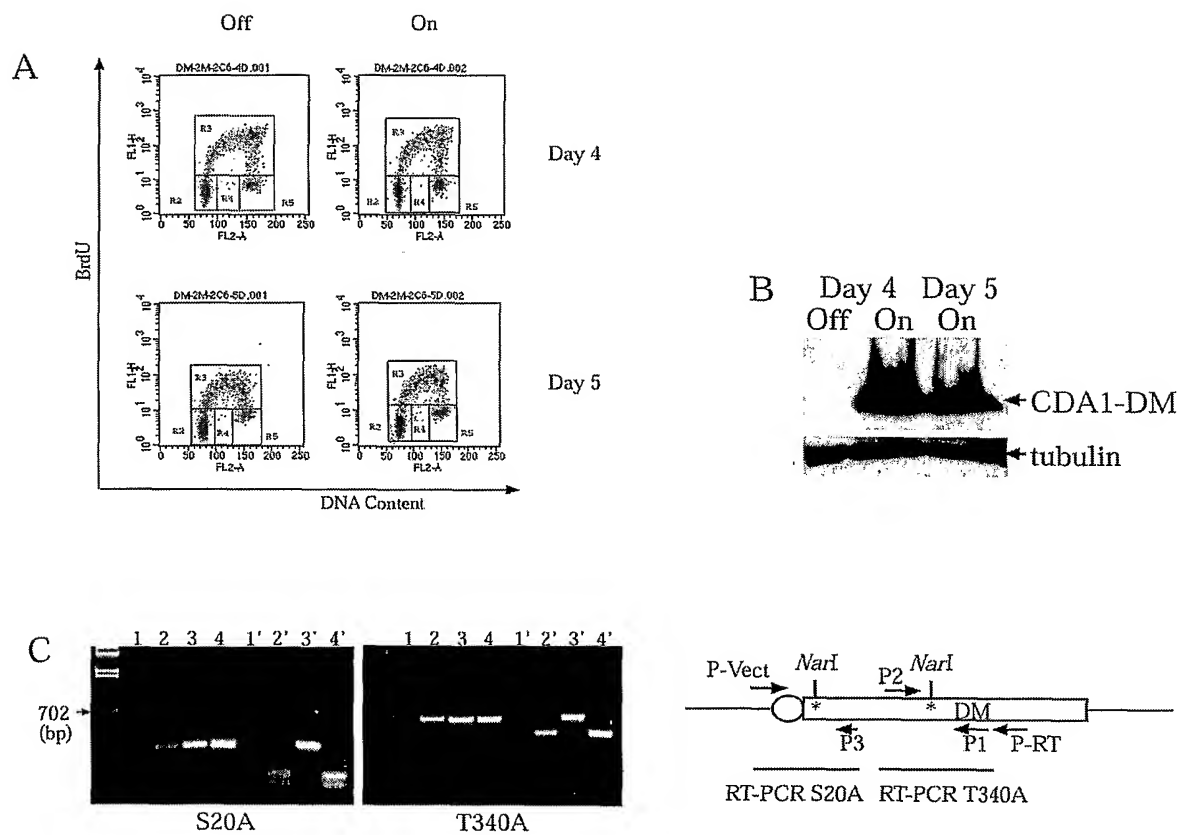


FIG 11

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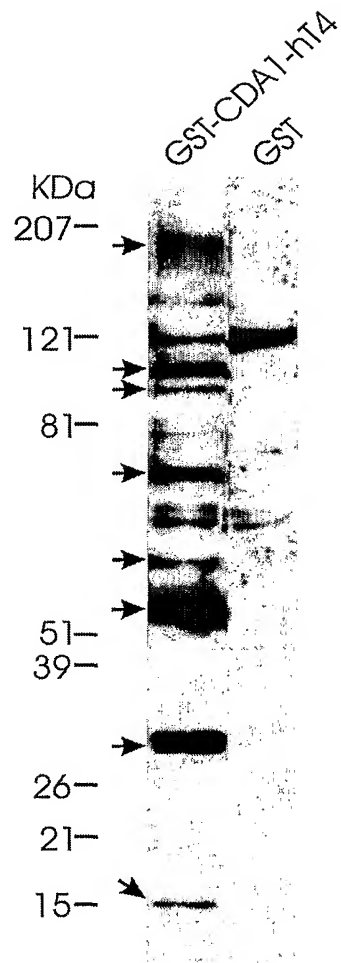


FIG 12

SEQUENCE LISTING

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and Uses Thereof

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35

40

45

Gln Glu Glu Thr Glu Ala Ala Gln Val Leu Ala Asp Met Arg Gly Val

50

55

60

Gly Leu Gly Pro Ala Leu Pro Pro Pro Pro Pro Tyr Val Ile Leu Glu

65

70

75

80

Glu Gly Gly Ile Arg Ala Tyr Phe Thr Leu Gly Ala Glu Cys Pro Gly

85

90

95

Trp Asp Ser Thr Ile Glu Ser Gly Tyr Gly Glu Ala Pro Pro Pro Thr

100

105

110

Glu Ser Leu Glu Ala Leu Pro Thr Pro Glu Ala Ser Gly Gly Ser Leu

115

120

125

Glu Ile Asp Phe Gln Val Val Gln Ser Ser Ser Phe Gly Gly Glu Gly

130

135

140

Ala Leu Glu Thr Cys Ser Ala Val Gly Trp Ala Pro Gln Arg Leu Val

145

150

155

160

Asp Pro Lys Ser Lys Glu Glu Ala Ile Ile Ile Val Glu Asp Glu Asp

165

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Glu Asp Glu Arg Glu Ser Met Arg Ser Ser Arg Arg Arg Arg Arg

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Arg Arg Arg Lys Gln Arg Lys Val Lys Arg Glu Ser Arg Glu Arg Asn

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Lys Arg Lys Phe Ile Gln Met Arg Arg Pro Phe Leu Glu Arg Arg Asp

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Leu Ile Ile Gln His Ile Pro Gly Phe Trp Val Lys Ala Phe Leu Asn

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265

270

His Pro Arg Ile Ser Ile Leu Ile Asn Arg Arg Asp Glu Asp Ile Phe

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280

285

Arg Tyr Leu Thr Asn Leu Gln Val Gln Asp Leu Arg His Ile Ser Met

290

295

300

Gly Tyr Lys Met Lys Leu Tyr Phe Gln Thr Asn Pro Tyr Phe Thr Asn

305

310

315

320

Met Val Ile Val Lys Glu Phe Gln Arg Asn Arg Ser Gly Arg Leu Val

325

330

335

Ser His Ser Thr Pro Ile Arg Trp His Arg Gly Gln Glu Pro Gln Ala

340

345

350

Arg Arg His Gly Asn Gln Asp Ala Ser His Ser Phe Phe Ser Trp Phe

355

360

365

Ser Asn His Ser Leu Pro Glu Ala Asp Arg Ile Ala Glu Ile Ile Lys

370

375

380

Asn Asp Leu Trp Val Asn Pro Leu Arg Tyr Tyr Leu Arg Glu Arg Gly

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390

395

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Ser Arg Ile Lys Arg Lys Lys Gln Glu Met Lys Lys Arg Lys Thr Arg

405

410

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450

455

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465

470

475

480

Asn Pro Asp His Asn Glu Val Pro Asn Asn Glu Thr Thr Asp Asn Asn

485

490

495

Glu Ser Ala Asp Asp His Glu Thr Thr Asp Asn Asn Glu Ser Ala Asp

500

505

510

Asp Asn Asn Glu Asn Pro Glu Asp Asn Asn Lys Asn Thr Asp Asp Asn

515

520

525

Glu Glu Asn Pro Asn Asn Asn Glu Asn Thr Tyr Gly Asn Asn Phe Phe

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535

540

Lys Gly Gly Phe Trp Gly Ser His Gly Asn Asn Gln Asp Ser Ser Asp

545

550

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Ser Asp Asn Glu Ala Asp Glu Ala Ser Asp Asp Glu Asp Asn Asp Gly

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570

575

Asn Glu Gly Asp Asn Glu Gly Ser Asp Asp Asp Gly Asn Glu Gly Asp

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590

Asn Glu Gly Ser Asp Asp Asp Asp Arg Asp Ile Glu Tyr Tyr Glu Lys

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Val Ile Glu Asp Phe Asp Lys Asp Gln Ala Asp Tyr Glu Asp Val Ile

610

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620

Glu Ile Ile Ser Asp Glu Ser Val Glu Glu Glu Gly Ile Glu Glu Gly

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640

Ile Gln Gln Asp Glu Asp Ile Tyr Glu Glu Gly Asn Tyr Glu Glu Glu

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Gly Ser Glu Asp Val Trp Glu Glu Gly Glu Asp Ser Asp Asp Ser Asp

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Leu Glu Asp Val Leu Gln Val Pro Asn Gly Trp Ala Asn Pro Gly Lys

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Arg Gly Lys Thr Gly

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU01/01418

A. CLASSIFICATION OF SUBJECT MATTERInt. Cl. ⁷: C12N 15/12; C07K 14/435, 14/46, 16/18; A61K 38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See databases below.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Genbank, EMBL, PDB nucleic acids, GenPept, Swiss-Prot, TREMBL, PIR - SEQ. ID. NOs: 1 and 2

DGENE - SEQ. ID. NO: 2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO A 01/53312 (HYSEQ, INC) 26 July 2001. See SEQ. IDs.	1-29
P, X	CHAI, Z. et al. SET-related cell division autoantigen-1 (CDA-1) arrests cell growth. The Journal of Biological Chemistry, September 2001, Vol. 276, No. 36, pages 33665-33674. See entire document, in particular Figure 2.	1-29
P, X	EP 1085024 A (HELIX RESEARCH INSTITUTE) 21 March 2001. See SEQ. IDs.	1-29

☒ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

7 January 2002

Date of mailing of the international search report

17 JAN 2002

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU01/01418

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	OZBUN, L.L. et al. Identification of differentially expressed nucleolar TGF- β 1 target (DENTT) in human lung cancer cells that is a new member of the TSPY/SET/NAP-1 superfamily. Genomics, April 2001, Vol. 73, pages 179-193. See entire document, in particular Figure 6.	1-29
P, X	GenPept Accession Number AAH01566 submitted 21 December 2000 by Strausberg R. See entire sequence.	1-29
P, X	EMBL Accession Number AF273046 submitted 31 May 2000 by Eichmuller S. et al. See entire sequence.	1-29
X	GenPept Accession Number BAA34802 submitted 8 June 1998 by Ueki N. See entire sequence.	1-29
X	EMBL Accession Number AB015345 submitted 8 June 1998 by Ueki N. See entire sequence.	1-29
X	UEKI, N. et al. Selection system for genes encoding nuclear-targeted proteins. Nature Biotechnology, 1998, Vol. 16, pages 1338-1342. See entire document.	1-29
X	EP 1033401 A (GENSET) 6 September 2000. See SEQ. IDs.	1-29

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU01/01418

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos : **30-34**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
These claims refer to antagonists of cell division autoantigen (CDA). The scope of the term antagonist is broad and may include antibodies, proteins, nucleic acids, carbohydrates, synthetic inhibitors and any other molecules which bind to CDA and modulate CDA activity. To search the entire scope of the claims is not economically feasible.
3. ☐ Claims Nos :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU01/01418

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	01/53312	AU	27284/01	AU	22924/01
		AU	25955/01	AU	25965/01
		WO	01/53500	WO	01/53515
		WO	01/53466	WO	01/53485
EP	1085024	JP	2000050881	JP	2000050882
		WO	99/64455	WO	99/64454
EP	1033401	JP	2001269182		
					END OF ANNEX